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A. I. PEROLD: Ondersoekings omtrent Moskonfyt

PRYS 1s.

NASIONALE PERS, BEPERK, KAAPSTAD.

Elke bydrae wat gedruk word verskyn as 'n afsonderlike aflewering, uitgenome in spesiale gevalle.

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Stukke vir opname en korrespondensie word geadresseer aan DR. W. BLOMMAERT, Uniwersiteit, Stellenbosch.

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Ondersoekings Omtrent Moskonfyt.

Deur Dr. A. I. PEROLD, Professor vir Wynbou en Enologie aan die Uniwersiteit van Stellenbosch.

(A summary in ENGLISH by the author appears at the end of the article.)

Onder die benaming ,,Moskonfyt'' word verstaan 'n min of meer gekleurde, dikvloeibare stroop, wat verkry is deur vars mos of druiwesap in die ope lug te kook tot dit dik genoeg is. Tot voor 'n tiental jare was feitlik al die moskonfyt in ons land gemaak deur die vars mos in 'n oop pot of ketel van koper of yster oor 'n direkte vuur te kook. Hierby ontstaan daar, by die begin van die kook, 'n laag skuim wat met 'n skuimspan afgeskep word. Namate die konsentrasie styg, klim ook die kookpunt ['n moskonfyt van 68° Brix het gekook op ca. 106°C. of 222.8°F.] en word die vloeistof donkerder van kleur, terwyl hy natuurlik ook meer dikvloeibaar word. Weens die direkte aanraking tussen die vuur en die pot, vind daar teen die boom en kante van die pot 'n lokale oorverhitting plaas, wat die *karamelisasie* van 'n klein gedeelte van die suiker bewerkstellig, waardeur die moskonfyt sy bruin kleur en 'n deel van sy geur kry. Dis moontlik dat ook die oorverhitting van organiese sure en suursoute in die mos hiertoe mag bydra.

In die laaste tyd word moskonfyt ook gekook deur stoom in die mos te lei of deur 'n geslote stoompyp in die vorm van 'n slang in die mos te plaas en daar stoom deur te laat gaan. Hier sal daar geen lokale oorverhitting plaasvind nie, en gevolglik sal die moskonfyt ligter van kleur wees as by die eersgenoemde manier van kook die geval was. Daar ook hier die kokende vloeistof aan die ope lug blootgestel is, sal die aldus gemaakte moskonfyt effens lig-bruin van kleur wees en ook 'n kooksmaak hê. Hy sal dus die eienskappe van die eersgenoemde soort moskonfyt in 'n flouer graad besit.

Die derde manier om mos te konsentreer is om hom in vakuumpotte te kook op 'n betreklik lae temperatuur maar onder 'n baie geringe druk. Volgens CRUESS (1),* bls. 406—408, sal druiwestroop onder 'n 24—26 duim vakuum (= 609.6—660.4 mm. vakuum of 150.4—99.6 mm. druk) op ongeveer 150°F. tot 135°F. (= 65.5°C. tot 57.2°C.) kook, en onder 29 duim vakuum (= 736.6 mm. vakuum of 23.4 mm. druk) op ongeveer 85°F. of 29.4°C. Onder 28 duim vakuum sal die stroop op ongeveer 40°C. kook. By Stellenbosch-stasie het mnr. Winshaw voor enige jare so 'n installasie opgesit van die tiepe wat onder 24—26 duim vakuum werk. Die produk is 'n taamlik liggekleurde stroop wat gewoonlik tot omtrent 68° Brix gekonsentreer word en „Grape Syrup" of Druiwestroop genoem word. Myns insiens moet laasgenoemde produk ook voortaan „druiwestroop" genoem word om dit te onderskei van die eintlike moskonfyt wat volgens die eerste of twede metode van kook berei is en in sy kleur, aroma en smaak baie verskil van „druiwestroop."

Daar is 'n vierde manier om mos te konsentreer, en dit is die teenoorgestelde van die voorgaande stelsels, naamlik deur die toepassing van groot koue. CRUESS (1), blss. 412—413, beskryf kortliks die *Gore*-proses vir die bereiding van druiwestroop deur bevriesing. Die helder mos word bevries op 10—15°F. tot 'n vaste massa, wat deur 'n ysbreekmasien fyn gemaak word, waarna die stroop van die ys geskeie word deur 'n sentrifuge. Hierdie stroop word weer bevries, en wel op 0—10°F., wanneer 'n pap met yskristalle verkry word wat gesentrifugeer word en nou 'n stroop van ca. 55° Balling lewer. Hierdie stroop is van die allerhoogste kwaliteit, behalwe dat dit te dun is om teen spontane gisting gevrywaar te bly as dit nie gesteriliseer word nie.

Hierdie verskillende produkte word vir verskillende doeleindes gebruik. Druiwestroop word hier meestal gebruik om sekere soetwyne te maak. Dit kan egter ook vir die aanmaak van heerlike alkohol-vrye dranke gebruik word, veral as die temperatuur in die vakuumpotte nie te hoog gegaan het nie. Volgens OTTAVI (2), bls. 267—268, het Fratelli Favara in Mazzara del Vallo (Sisielië) reeds in 1888 daarin geslaag om 'n druiwestroop in vakuumpotte te kook op 'n temperatuur wat altyd laer as 40°*C.* *of* 104°*F.* was, en tot 'n gemiddelde konsentrasie van ca. 70° Brix (S.G. 1.35).

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* Die nommer agter die naam van die outeur refereer na die ooreenkomende nommer van die literatuuroorsig.

Die eintlike moskonfyt word meestal as jam saam met brood geëet, alhoewel dit ook vir die maak van seker soorte soetwyn kan gebruik word. By die bereiding van die beroemde Malaga- en Marsala-wyne word moskonfyt ook gebruik. Dit word in Malaga in groot oop, taamlike diep, koniese ysterpotte gekook tot dit goed dik en donker van kleur is, en ,,arrope'' genoem. In Marsala word dit in taamlike vlak, vertinde koperpotte tot ongeveer een-vyfde van sy oorspronklike volume gekook, en ,,mosto cotto'' genoem.

In verband met moskonfyt en sy bereiding, is dit van belang om daarop te wys dat die druifsoort wat die mos gelewer het 'n groot invloed uitoefen op die kwaliteit van die moskonfyt. Fransdruif gee die geurigste en lekkerste moskonfyt, terwyl Hanepoot ook 'n lekker moskonfyt lewer. In die eersgenoemde geval het ons te doen met 'n mos wat 'n lae totale suurgehalte besit—gewoonlik net omtrent $4^0/_{00}$ berekend as wynsteensuur. Die druiwe moet verder goed soet wees. Om 'n liggekleurde moskonfyt te kry, moet ons die vars mos met swaweldioksied of kaliummetabisulfiet (die natrium- en kalsiumverbindinge is net so goed) behandel, en die helder mos in 'n *dun* laag (sê 6 duim diep) *vinnig* kook in 'n koperketel tot dit dik genoeg is. In plaas van koper kan aluminium, tin, vertinde of versilwerde koper gebruik word.

By die bereiding van moskonfyt is die volgende vier punte van die allergrootste belang: Bewaring van mos, ontsuring van mos, konsentrasie waarop die stroop moet gekook word en voorkom van latere versuikering van die stroop. Die eerste drie van die genoemde punte sal hier bespreek word, en die resultate van my eie ondersoekings in 1922 hieromtrent meegedeel word, terwyl die laaste punt nie nou sal behandel word nie, maar die onderwerp van verdere ondersoek sal vorm, waaromtrent later sal berig word.

I. DIE BEWARING VAN MOS.

Daar dit tyd neem om die moskonfyt te kook, en vars mos nie vir 'n onbeperkte tyd beskikbaar is nie, moet ons die mos 'n tyd lang bêre en intussen die gisting belet. Dit kan die beste gebeur deur swaweldioksiedgas in die vars mos te pomp, wat hom dan in 'n stukvat of sementtenk bevind, waar hy van die lug kan afgesluit word. Volgens prof. VENTRE (3), bls. 23,

is die gebruik van swaweldioksied, om mos stil te hou, reeds voor meer as 100 jaar aanbeveel, en ook in die teenswoordige doses, 1—2 g. per lit.: 5—6 kg. swawel (verbrand) per 50—60 hl. mos [vgl. PARMENTIER (4)]. In plaas van genoemde gas kan ons ook die vloeibare swaweldioksied of 'n oplossing van swaweldioksied in water of 'n metabisulfiet gebruik.

Die belangrike vraag is *hoeveel* swaweldioksied ons per lêer mos nodig sal hê om hom vir 'n byna onbeperkte tyd van gisting te bewaar. Dis bekend dat 'n deel van die swaweldioksied aan die suiker in die mos gemies gebind word, en so te sê ophou om 'n antiseptiese invloed op die gis-selle uit te oefen. Dis feitlik net die vry geblewe swaweldioksied wat sodanige antiseptiese invloed uitoefen. Hieruit blyk dus dat ons meer swaweldioksied of metabisulfiet moet byvoeg as in 'n waterige oplossing voldoende sou wees om die spruiting van die gis-selle te belet, en dat ons meer sal moet byvoeg namate die mos soeter is. Die temperatuur is ook van belang, daar meer nodig is by warm as by koel of koue weer. Verder is die tyd van bewaring van belang, weens die gestadig voortgaande binding van swaweldioksied deur die suiker, en weens die oksidasie van swawelige suur tot swawelsuur waar die lug toegang tot die mos kry, soos deur die porieë van 'n stukvat se hout geskied. KERP (6) [volgens LAFAR (5), Bd. V, 446] het bewys dat die swawelige suur net deur die dekstrose gebind word. Volgens DUPONT en VENTRE (7) [uit LAFAR (5), V, 447] is die ewewigstoestand in mos ná 8—14 dae bereik. Waar hul 500 mg. swaweldioksied per liter mos bygevoeg het, was daar ná 8 dae net nog 140 mg. vrye swaweldioksied per liter mos aanwesig. Waar 100 mg. bygevoeg was, was daar ná 8 dae nog 8 mg. vry.

Volgens LINOSSIER (8) [uit LAFAR (5), V, 448] word die gisting van mos volkome onderdruk deur 'n byvoeging van 0.675‰ SO_2, d.w.s. 675 mg. SO_2 per liter of 388 g. SO_2 of 776 g. (=1.71 lb.) van 'n metabisulfiet met 50% SO_2 per lêer mos. LABORDE (9), I, 107, gee 0.500—1 g. SO_2 per liter mos aan as die nodige dosis om die gisting te belet. Dit staan gelyk met 288.5—577 g. SO_2 of 577—1154 (= ca. $1\frac{1}{4}$—$2\frac{1}{2}$ lb.) metabisulfiet @ 50% SO_2 per lêer mos. Volgens CRUESS (1), bls. 414, is 1.2—1.5 g. SO_2 per liter nodig in warm streke en 0.75—1.00 g. per liter in koeler streke om die gisting van mos vir 'n onbepaalde tyd te belet.

EIE ONDERSOEKINGS.

(a) *Groendruif-mos* met S.G. 1.106 by 25°C., wat 'n totale ekstrakgehalte van 26.0° Brix (of Balling) betekent en met 'n totale suurgehalte van 5.38°/₀₀,* is op die 6e Maart direk van die doppe afgeloop en in 'n oop sementgat gepomp. Hier is by 3½ lêer mos 3½ lb. kaliummetabisulfiet gevoeg namate die mos ingepomp is. Die dosis was dus 1 lb. metabisulfiet per lêer mos of ca. 393 mg. SO_2 per liter mos. Van die afgesakte helder mos in 'n oop sementkuip is moskonfyt gekook tot die 10e Maart, toe die mos begin werk het. Hier was die gisting dus vir 4 dae teëgehou deur genoemde dosis van metabisulfiet.

(b) *Hanepoot-mos* direk afgeloop van vars doppe op 21.4.22, met S.G. 1.0976 ($\frac{17.5°C.}{17.5°C.}$) wat 'n totale ekstrakgehalte van 23.17° Brix of 25.4 g. per 100 c.c. beteken, is direk in 'n stukvaatjie van ca. 220 gellings inhoud gepomp en daarin is 6 lb. kalium-metabisulfiet opgelos, en die vaatjie is met mos gevul en toegemaak. Hier was by die begin dus byna 3½ lb. kaliummetabisulfiet per lêer of 1.362 g. SO_2 per liter mos bygevoeg. Op 27.4.22, dus 6 dae later, is die stil helder mos ontleed.

Bepaling van SO_2

Dit is geskied deur die mos met $\frac{N}{50}$ J. koud te titreer met setmeeloplossing as indikator. Daar die aanwesige suiker hinderend werk, was die end van die titrasie gekome geag wanneer die blou kleur 'n paar sekondes lank stand gehou het.

20 c.c. mos het gebruik 17.8, 18.2, 17.9, 17.8 c.c. $\frac{N}{50}$ J. Dus bevat 1 liter mos 17.8 x 0.032 = 0.5696 g. vrye SO_2. Die totale SO_2-gehalte van die mos is bepaal volgens die gebruiklike jodometriese metode [vgl. WILEY (14), Bd. III, 804].

20 c.c. mos het 37.1 c.c. $\frac{N}{50}$ J. gebruik; dus het die mos in totaal 1.1872 g. SO_2 per liter bevat, en was die vrye swaweldioksied ná 6 dae nog byna net so veel as dié wat gebonde was.

Bepaling van Totale Suur.

20 c.c. mos het gebruik 9.8 c.c. Bariet (f=0.1478).
20 c.c. mos het gebruik 9.8 c.c. Bariet (f=0.1478).
*Totale suurgehalte=5.43°/₀₀

* Die totale suurgehalte word hier altyd aangegee as so veel gram wynsteensuur per liter.

Bepaling van Soortelike Gewig en Ekstrak.

S.G. 1.0989 ($\frac{17.5°C.}{17.5°C.}$), dus 23.48° Brix, en 25.80 g. totale ekstrak per 100 c.c. mos. Die byvoeging van die metabisulfiet kon die ekstrak met 0.3 g. per 100 c.c. verhoog, dus kon ons hier 25.4 + 0.3 = 25.7 g. per 100 c.c. verwag het, wat goed ooreenstem met die gevonde 25.8 g. per 100 c.c.

Op 7.5.22 was die mos nog stil, maar reeds die volgende dag was hy hard aan die werk. Ek het toe dadelik sy SO_2-gehalte bepaal, en gevind 281.6 mg. vrye SO_2 per liter, en 800.0 mg. totale SO_2 per liter.

Uit die voorgaande blyk dus dat die bogenoemde syfers van LINOSSIER en LABORDE *geen dodelike doses aangee* nie, en dat hul nie in alle gevalle toereikend is om 'n gisting vir 'n lang tyd te belet nie. Die syfers deur CRUESS (1) vir warm streke aanbeveel—wat eers by die opstel van hierdie berig in my hande gekom het—word deur hierdie proef gesteun. Hier het ca. 1.362 g. [en seker > 1.1872 g.] SO_2 per liter mos die gisting vir 16 dae belet, maar toe het 'n flukse gisting begin en die mos het droog gewerk. Die helder mos het hier net so op die afsaksel in die vat bly lê. Mos wat lank moet bewaar word eer daar moskonfyt van gekook word, moet, ná dit afgesak is, van die afsaksel geskeie word. Hiervoor sal dit dan die beste wees om by die vars mos ½ lb. metabisulfiet per lêer of 0.2 g. SO_2 per liter mos te voeg, waardeur die gisting lank genoeg sal gekeer word dat die mos helder kan afsak. Tap nou die helder mos af in 'n stukvat of sementtenk en gee hom 4 lb. metabisulfiet (@ 50% SO_2) per lêer of 1.6 g. SO_2 per liter mos. Dit is vir Suid-Afrikaanse toestande bedoel, en ek had reeds tot hierdie aanbeveling besluit voor ek nog die syfers van Cruess gesien had, wat 1.5 g. SO_2 per liter mos as hoogste dosis aanbeveel. In hoe verre die deur my aanbevole dosis SO_2 onder alle omstandighede as 'n genoegsame dosis beskou kan word om alle gisting vir 'n onbepaalde tyd te belet, sal die tyd ons nog moet leer. As daar 'n dodelike dosis SO_2 vir wyngiste bestaan, dan sal dit waarskynlik nog heelwat hoër wees as die dosis wat ek hier aanbeveel om die gisting te belet.

II. DIE TOTALE SUURGEHALTE VAN MOSKONFYT EN DIE VERMINDERING DAARVAN.

Waar moskonfyt vir eetdoeleindes gekook word, daar is dit wenslik om sy natuurlike suurgehalte af te bring. Waar hy vir versnit met wyn gebruik word, daar is dit nie so noodsaaklik nie, alhoewel dit soms beter mag wees. Waar moskonfyt, en veral druiwestroop, gebruik word om alkoholvrye drank van te maak, of ook wel alkoholiese drank, daar moet die suurgehalte van die mos of stroop nie verminder word nie.

Die doeltreffendste manier om die moskonfyt se totale suurgehalte te verminder is om dit met die mos te doen en nie met die klaar stroop nie. Die gewone middel wat hiervoor gebruik word is kalk. Ons boere gebruik gewoonlik gebluste kalk, so na skatting. Die gevaar hierby is dat te veel kan gebruik word. Gebeur dit en word die mos alkalies gemaak, dan sal sulke mos 'n baie donker en bitter moskonfyt gee wat niks werd is nie. So 'n geval is onder my aandag gekom. Die veiligste is dus fyngemaalde kalkklip of marmer. Hier bestaan daar natuurlik geen gevaar dat die mos alkalies kan word as te veel gebruik word nie.

Prof. J. VENTRE, l.c., bls. 26, beveel aan om, waar ons 'n stroop wil maak wat met ,,Golden Syrup'' kan kompeteer, al die suur in die mos met Ca-karbonaat te neutraliseer, ná 'n behandeling met dierkool om die mos te ontkleur en 'n daaropvolgende breisel met 10—15 g. gelatien of 100—150 c.c. vars bloed per hektoliter [gelyk aan ca. 2—3 onse gelatien per lêer of 1 gelling vars bloed vir 8 lêers] mos om die mos weer skitterend helder te maak. Die neutralisasie sal gedurende die kook voortgaan. Hy beveel aan om net so veel gram Ca-karbonaat te gebruik as die mos totale suur, berekend as swawelsuur, bevat, aangesien 98 g. swawelsuur geneutraliseer word deur 100 g. kalsiumkarbonaat.

Die geurigste en lekkerste moskonfyt moet nog 'n seker hoeveelheid suur bevat.

EIE PROEWE IN 1922.

My eerste monster moskonfyt is in 1922 gekook van groendruifmos met 'n totale suurgehalte van 5.38‰ en 'n ekstrakgehalte van 25.5° Brix (of Balling). By hierdie mos is die aand voor dit gekook is 9 g. reine kalsiumhidraat per gelling

(=2 g. per lit.) gevoeg, wat 'n totale suurvermindering van 4 g. wynsteensuur per liter sal veroorsaak, aangesien 74 g. CaO_2H_2 sekuur 150 g. wynsteensuur sal neutraliseer. Die moskonfyt is gekook tot 'n konsentrasie van 67.5° Brix. Sy totale suurgehalte was 6.38°/₀₀, dus net 1°/₀₀ hoër as dié van die mos. Hy was taamlik lekker, maar te dun, en het dan ook later aan die werk geraak.

My laaste lot moskonfyt is in 1922 gekook van Hanepootmos met 5.43°/₀₀ totale suur en 23.48° Brix ná 'n byvoeging van 3½ lb. metabisulfiet per lêer mos. By 20 gellings mos is 1 lb. gemaalde kalkklip met 70.7% $CaCO_3$, dus 3.53 g. $CaCO_3$ per liter mos, gevoeg en saam gekook. Die moskonfyt is ingekook tot 69.7° Brix [S.G. 1.3490 $(\frac{17.5°C.}{17.5°C.})$] en had 5.52°/₀₀ totale suur. Dit was 'n baie lekker moskonfyt. Sy totale suurgehalte was ongeveer gelyk aan dié van die oorspronklike mos. As al die Ca-karbonaat by die kook deur die aanwesige sure gebind was, dan sou daardeur 'n suurvermindering van 5.29°/₀₀ veroorsaak geword het, en sou die mos net nog 0.23°/₀₀ totale suur behou het.

Daar 20 gellings mos 4.5 gellings moskonfyt gegee het, was 1 gelling moskonfyt uit 4.4 gellings mos ontstaan, en moes die totale suurgehalte van die moskonfyt 4.4 x 0.23°/₀₀ = 1.02°/₀₀ bedra het i.p.v. 5.52°/₀₀. Gevolglik bevat die moskonfyt 4.50°/₀₀ meer totale suur as te verwagte was, en het die mos $\frac{4.50}{4.4}$ = 1.01°/₀₀ te veel totale suur behou. Dus is daar maar 5.49—1.01 = 4.28°/₀₀ totale suur van die mos deur die kalkklip geneutraliseer i.p.v. 5.29°/₀₀, of m.a.w. het daar net $\frac{4.28 \times 100}{5.29}$ = 80.9% van die bygevoegde kalk in reaksie getree met die aanwesige sure.

Volgens die hier beskrewe ondervinding, meen ek dat die gewenste hoeveelheid Ca-karbonaat om by ons vir moskonfyt te voeg ongeveer 3½—4 lb. per 100 gellings sal wees. Dit veronderstel kalkklip met 100% $CaCO_3$ en mos met 5—6°/₀₀ totale suur. As die kalkklip x% $CaCO_3$ bevat, dan gebruik ons $\frac{100}{x}$ x (3½ tot 4) lb. kalkklip per 100 gellings mos. As ons uit mos 'n soort „Golden Syrup" i.p.v. moskonfyt wil maak, dan sal ons ongeveer dubbel die genoemde hoeveelhede moet gebruik, want ons sal aanmerklik meer moet neem as teoreties nodig is om al die aanwesige sure te neutraliseer.

III. DIE BESTE KONSENTRASIE VIR MOSKONFYT.

Aanhalings uit die literatuur wat hierop betrekking het en wat vir my toeganklik was:

1. MERZ (DAL PIAZ) (10), bls. 122: „Bei einem Mindestzuckergehalt von 50% (Hektolitergewicht = 129 Kg., Dichte = 33° Baumé oder 60° Balling) ist die Haltbarkeit gesichert und sind dann selbst bei der Lagerung in wärmeren Räumen keinerlei Gärungserscheinungen zu befürchten."

2. Prof. J. VENTRE (3), bls. 25: „Si on veut être assuré, contre tout départ spontané de fermentation, la concentration devra être poussée jusqu' à 36°B. au moins. On se trouve alors en présence d'un véritable sirop de raisin dont la conservation sera pour ainsi dire indéfinie."

3. DUBOURG (11) het uit 'n baie soet Sauternes-wyn van 1893 giste geïsoleer wat gisting veroorsaak het in 'n vloeistof met tot 80% inwertsuiker. Hul is geïsoleer uit wyne afkomstig van moste wat tot 600 g. suiker per liter bevat het. Hul laat almal die lewulose gouer gis as die dekstrose (dus die omgekeerde van wat gewoonlik gebeur!), maar skeie geen sukrase uit nie en kan gevolglik sakkarose nie direk laat gis nie.

4. BOKORNY (12) het baie persgis („Presshefe") met hoogpersentige oplossings van druiwesuiker en rietsuiker saamgebring. By 48.8% druiwesuiker was daar nog 'n flukse gisting, maar by 48.8% rietsuiker amper niks. Die inwersie was hier dus byna nul. By 58.8% druiwesuiker was die gisting nog sterk, maar by 58.8% rietsuiker was dit nul, wat net aan 'n gebrek aan inwersie kan te wyte wees. By die konsentrasie van 74% het geen een gegis nie. Die inwertase hou dus ongeveer by 48% suiker op om te werk, en die simase eers bo 58.8%.

5. LAFAR (5), Bd. V, blss. 70—71: „Die Haltbarkeit des *Gelees, Fruchtsirupe, Marmeladen,* u.s.w. beruht ebenfalls auf ihrer osmotischen Wirkung, die das Wachstum hinzutretender Pilzkeime verhindert. . . . . Von der Firma *Fratelli Favara* in Mazzara del Vallo in Sizilien wird Traubensaft nach vorausgegangenem Filtrieren im Vacuumapparat bei ungefähr 40° auf ein Viertel des ursprünglichen Volumens eingedampft. Trotzdem dieser sirupartige *konzentrierte Most* von ca. 62 Proz. Zuckergehalt nicht steril ist, sondern u.a. lebensfähige Hefen, darunter

regelmässig *Sacch. Apiculatus,* enthält, bleibt er doch unverändert, und geht erst nach Verdünnung von selbst in Gärung über. . . . . Ein Zuckergehalt von 57—60 Proz. genügt also, Gärung oder anderweitige Zersetzungen zu verhindern."

6. KÖNIG (13), Bd. III, 2. Teil, S. 916, gee die volgende ontledings van sommige vrugtestrope :—

	Getal ontledings.	S.G.	Ekstrak Gew. %	Inwertsuiker Gew. %	Rietsuiker Gew. %	Droë ekstrak Gew. %	Totale suur as Appelsuur Gew. %
Himbeersirup - -	45	1·3227	66·26	22·39	42·25	1·82	0·598
Erdbeersirup - -	7	1·3075	62·82	23·55	37·72	1·60	0·315
Kirschsirup - -	6	1·3387	68·95				0·401

7. OTTAVI (2), bls. 268 : „Il concentrato del Favara presenta una densitá media di 1,35 [=ca. 70° Brix—A.I.P.] e offre questa costituzione :—

	per ettolitro	*per quintale**
Glucosio	Kg. 90.000	Kg. 66.66
Acidi	Kg. 2.400	Kg. 1.77 "

By 70° Brix is die totale ekstrakgehalte 94.5 g. per 100 c.c.

8. CRUESS (1), bls. 409, sê : „We have found that syrup of 65° or 66° Balling will soon ferment and become mouldy, but that syrup of 70° Balling will keep perfectly."

Uit hierdie aanhalings blyk dat die aanbevole konsentrasies loop van 60°—70° Brix, as ons die geval van gisting by 80% inwertsuiker, deur Dubourg genoem, opvat as betekenende 80 g. per 100 c.c., wat ongeveer 61.6 gew. % inwertsuiker sal beteken.

By die kook van moskonfyt moet ons in die eerste plaas sorg dat hy *dik genoeg gekook word om nie later aan die werk te raak nie.* Tegelykertyd moet ons daarna strewe om 'n latere versuikering, d.w.s. uitkristallisasie van suiker (dit sal die dekstrose wees) en wynsteen, te voorkom. Ek het by die begin van my ondersoek gehoop dat daar 'n sekere konsentrasie gevind sal word, waarop nòg die een nòg die ander sal plaasvind. Uit my ondersoek is nou ongelukkig gebleke dat daar gevalle kan voorkom waarby gisting sowel as versuikering plaasvind.

* Dit is 100 Kg. (A.I.P.).

Eie Ondersoek.

(a) *Gevalle waar moskonfyte spontaan aan die werk gegaan het.*

Oorsprong.	S.G. by $\frac{17.5°C.}{17.5°C.}$	Grade Brix.	Totale ekstrak per 100 c.c.	Inwert-suiker per 100 c.c.	Droë ekstrak per 100 c.c.	Totale suur as wynsteen-suur per ‰.
1. Welgeval-len, 1922.	1·3107	63·5	83·2			
2. „	1·3319 (20°/20°)	67·2	89·5	79·7	9·8	6·83
3. „	1·3494	69·75	94·1	84·1	10 0	5·31
4. „	1·3398	68·2	91·3	81·7	9·6	
5. Stellenbosch Distillery, 1922.	1·3413	68·5	91·8	84·8	7·0	15·30

Nommers 3 en 5 het respektieflik 62.3 en 63.2 gew. % inwertsuiker bevat.

Hierdie moskonfyte het by kamertemperatuur in die laboratorium, meestal reeds gedurende die winter, aan die werk geraak. Aan die oppervlakte was 'n laag of lagie skuim aanwesig. Sodra 'n bottel aan die werk geraak het, is daar 'n watteprop opgesit. No. 3 gee die ontleding van moskonfyt A Welgevallen 1922 op 13.5.22 eer dit gegis het. No. 4 gee die ontleding van dieselfde moskonfyt op 14.11.22, dus 6 maande later, ná dit reeds 'n hele ruk swak aan die werk was. No. 5 is 'n druiwestroop wat in 'n vakuumpot gekook is, en op 11.4.22 ontleed is eer daar nog 'n gisting was. Die inwertsuiker is by Nos. 2, 3, 5 met Fehlingse oplossing volumetries bepaal, en grawimetries in geval van No. 4.

By No. 2 was 9 g. CaO_2H_2 per gelling mos(=2 g. per lit.) gebruik en die mos had oorspronklik 5.38°/₀₀ totale suur. By No. 3 was 1 lb. fyn gemaalde kalkklip (=0.707 lb. $CaCO^3$) per 20 gellings mos gebruik en die mos had oorspronklik 5.43°/₀₀ totale suur. By No. 5 was geen kalk gebruik nie en dus is sy hoë totale suurgehalte maklik te verstaan.

(*b*) *Moskonfyte wat nie aan die werk geraak het nie*:

1. Moskonfyt van I. S. Perold, P. A. Hamlet, van 1922 met S.G. 1.3570 $(\frac{17.5°C.}{17.5°C.})$ = 71.0° Brix, en 'n totale suurgehalte van 4.25°/₀₀.

2. Moskonfyt Welgevallen 1920 het nie gegis nie tot ná dit in die laboratorium in 1922 in 'n ander fles oorgegooi was. Sy s.g. op 17.5°C. per piknometer, was 1.3316=66.9° Brix.

Geen moskonfyt met 71° Brix of meer het aan die werk geraak nie.

(c) Moskonfyte met reingis geënt.

Ten einde vas te stel op watter konsentrasie gisting nog kan plaasvind, is die hier volgende proewe gemaak.

Moskonfyt A Welgevallen 1922 is langsaam ingedik tot 79.4° Brix, en hieruit is 6 konsentrasies gemaak. Van elke konsentrasie is ca. 8 c.c. in elk van 6 steriele proefbuisies met watteproppe gegooi en toe vir ¾ uur op 100° in 'n stoomsterilisator gehou. Vier weke later is hul in elke reeks met 4 aparte spruitende reingiste geënt, een waarvan gekweek was uit gistende moskonfyt met 63.5° Brix. Die entmateriaal was 'n platinaogie vol gistende vloeistof met die reingisselle. Die samestelling van die moskonfyte was soos volg.—

No. van Moskonfyt.	Grade Brix.	Inwertsuiker Gew. %.	Inwertsuiker per 100 c.c.	Totale suur ‰ as wynsteensuur.
I	79·4	71·1	100·4 g	7·00
II	76·68	68·7	95·6 g	6·67
III	74·14	66·4	91·3 g	6·36
IV	71·77	64·3	87·3 g	6·09
V	69·55	62·3	83·7 g	5·83
VI	67·45	60·4	80·3 g	5·70

Die konsentrasies II tot VI is uit I verkry deur 84.7 g. (=60 c.c.) van I met resp. 3, 6, 9. 12, 15 g. gedistilleerde water te vermeng, en hul samestelling is dienooreenkomstig uit dié van I bereken.

Voor die enting was die moskonfyt in die buisies pragtig helder en hul het stadigaan van bo af donkerder van kleur geword, wat skyn aan te duie dat daar na al die kook nog 'n aktiewe oksiderende ensiem aanwesig was. Die buisies is op 15.6.22 geënt, en tot nog toe, 7 maande daarna, het geen een van hul aan die gis geraak of troewel geword nie. Die res van Konsentrasie VI, wat in 'n ½ lit. botteltjie oorgebly het, het spontaan aan die werk geraak, terwyl dit nie met die hoër konsentrasies gebeur het nie. Hierdie eksperiment sal voortgesit word.

(*d*) *Gevalle van Versuikering.*

1. Moskonfyt van I. S. Perold, 1922, met 71.0° Brix het totaal versuiker in 'n halfgevulde ¼ lit. flessie, wat met 'n glasprop gesluit was.

2. Druiwestroop van Stellenbosch-Distillery, 1922, met 68.5° Brix het gedeeltelik versuiker.

3. Die Konsentrasies I—IV (79.4—71.77° Brix) in voorgenoemde eksperiment het totaal tot gedeeltelik versuiker waar hul in die ¼ lit. flessies oorgebly het. In die proefbuisies wat vir ¾ uur in die stoomsterilisator verhit geword is, het nêrens versuikering plaasgevind nie. Die kwessie van versuikering word verder ondersoek.

(*e*) *Gelyktydige versuikering en gisting.*

Dit het gebeur met die druiwestroop van die Stellenbosch Distillery, 1922, met 68.5° Brix. Hieruit blyk dus dat, onder seker omstandighede, versuikering *reeds* kan begin terwyl gisting *nog* plaasvind. By die begin van my ondersoek het ek gehoop dat daar 'n konsentrasie sal gevind word waarop geen gisting meer sal plaasvind nie, terwyl die stroop dan nog nie so hoog gekonsentreer sal wees om te kan versuiker nie. Uit genoemde geval is gebleke dat so'n konsentrasie nie bestaan nie. Ons moet die moskonfyt dus so dik kook dat hy in elk geval teen gisting gevrywaar sal wees, wat m.i. die geval sal wees op 'n konsentrasie van 71° Brix. Sulke moskonfyt is baie geneig om te versuiker, en my verdere ondersoek omtrent moskonfyt sal hoofsaaklik gaan oor die vraag hoe om moskonfyt tot 71° Brix te kook sonder dat hy later sal versuiker.

Opsomming.

1. Om mos vir die bereiding van moskonfyt of druiwestroop vir 'n onbepaalde tyd te kan bewaar sonder dat dit aan die werk sal raak moet by die vars mos 200 mg. SO_2 per liter (=¼ lb. SO_2 of ½ lb. metabisulfiet per lêer) gevoeg word om die mos helder te laat afsak. Die helder mos moet na omtrent 36 uur in 'n vat gepomp word, waar daar nou 1600 m. SO_2 per liter, gelyk aan 2 lb. SO_2 of 4 lb. metabisulfiet per lêer, mos bygevoeg word.

2. Ten einde die moskonfyt se totale suurgehalte taamlik laag te kry en hom lekker te laat smaak, beveel ek aan om $3\frac{1}{2}$—4 lb. fyn kalsiumkarbonaat by elke 100 gellings mos (namate dit gebruik word) te voeg en saam te kook. Ek veronderstel dat die mos omtrent 5—6‰ totale suur, berekend as wynsteensuur, bevat. Van fyngemaalde kalkklip moet $\frac{100}{x}$ x ($3\frac{1}{2}$ tot 4) lb. geneem word, waar x = persentasie $CaCO_3$ in die kalkklip.

3. Kook die moskonfyt tot 'n konsentrasie van 71° Brix of Balling bereik is. Dit beteken dat die moskonfyt 'n s.g. van 1.3572 sal hê op 17.5°C. of 63.5°F., en dat 1 gelling moskonfyt op hierdie temperatuur 13 lb. 9 os. sal weeg. Op 100°C. of 212°F. sal die stroop se s.g. ongeveer 1.3065 wees en sal 1 gelling stroop 13 lb. 1 os. weeg.

4. Sulke moskonfyt sal meesal versuiker. Hoe om dit te voorkom, vorm die onderwerp van my verdere ondersoek.

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Investigations about Moskonfyt.

By Dr. A. I. PEROLD, Professor of Viticulture and Oenology at the University of Stellenbosch.

In South Africa the term "moskonfyt" is used for a grape syrup that has been boiled in an open pot over a direct fire. It constitutes a light till dark brown syrup with a pleasant aromatic taste and flavour *sui generis*. It is commonly used for human consumption in the same way as Golden Syrup, but also serves for sweetening certain wines. It differs materially in colour and flavour from a grape syrup that has been obtained by boiling at a low temperature in a vacuum pan. In my opinion the term "moskonfyt" should not be applied to this latter type of syrup, which should be known and sold as "Grape Syrup."

The following points are of the greatest importance in the manufacture of Moskonfyt:—Preservation of juice or must, deacidification of must, concentration to which the syrup must be boiled, prevention of subsequent crystallisation of the syrup.

In this paper the first three points are discussed, and the results of my investigations thereanent during 1922 are communicated, whilst the last point forms the subject of further investigation, the results of which will be published in due course.

I. PRESERVATION OF MUST.

The most suitable preservative for this purpose is sulphurdioxide or a metabisulphite. The important point is the quantity to be used. LINOSSIER (8)* considers an addition of 675 mg. SO_2 per litre, and LABORDE (9), I, 107, considers 500—1,000 mg. SO_2 per litre as quite sufficient to prevent any fermentation. CRUESS (1), p. 414, states that the amount of sulphurdioxide necessary to prevent fermentation is 1,200—1,500 mg. per litre in hot localities, and in cooler localities 750—1,000 mg. per litre.

* The number behind the author's name refers to the corresponding number in the summary of the literature.

In my own experiments I found that the fermentation was delayed as follows:—

(*a*) *Greengrape-must* of 26.0° Brix (or Balling) with a total acidity of 5.38°/$_{00}$ (calculated as tartaric acid) was immediately given 1 lb. Pot. metabisulphite per leaguer or about 393 mg. SO_2 per litre, when fermentation set in after 4 days.

(*b*)† *Hanepoot-must* of 23.17° Brix was given about 3½ lbs. Pot. metabilsulphite per leaguer or 1,362 mg. SO_2 per litre in a closed, full cask, and started fermenting vigorously after 16 days, the result being a dry wine. On the 6th day it contained 569.6 mg. free and 1,187 mg. total SO_2 per litre. On the 17th day, when fermentation had actively started, it still contained 281.6 mg. free and 800 mg. total SO_2 per litre.

I therefore recommend the addition of ½ lb. meta-bisulphite (@ 50% SO_2) per leaguer or 200 mg. SO_2 per litre fresh must left in an open tank for settling. The clear supernatant liquid must be withdrawn after about 36 hours and immediately given a further dose of 4 lbs. metabisulphite per leaguer or 1,600 mg. SO_2 per litre. This is meant for South African conditions, but will probably answer anywhere.

II. DEACIDIFICATION OF MUST.

Moskonfyt should retain a certain acidity to be of good quality. Greengrape-must with a total acidity of 5.38°/$_{00}$ and total extract of 25.5° Balling was treated with 2 g. CaO_2H_2 per litre, and yielded a good moskonfyt with a total acidity of 6.38°/$_{00}$ at 67.5° Brix or Balling.

Hanepoot-must with a total acidity of 5.43°/$_{00}$ and total extract of 23.48° Brix was boiled with a finely-ground limestone, containing 70.7% $CaCO^3$ at the rate of 1 lb. per 20 gallons or 3.53 g. $CaCO_3$ per litre. It gave a very nice moskonfyt with a total acidity of 5.52°/$_{00}$ at 69.7° Brix. Thus only 80.9% of the added limestone entered into reaction.

I recommend the addition of 3½—4 lbs. calcium carbonate to every 100 gallons of must with 5—6°/$_{00}$ total acidity, the two being boiled together.

† Hanepoot is the same as Muscat of Alexandria.

III. THE BEST CONCENTRATION FOR MOSKONFYT.

The following concentrations have been recommended as being sufficiently high to prevent the syrup undergoing any spontaneous fermentation :—

1. MERZ (DAL PIAZ) (10), p. 122 : at least 50% sugar=33° Bé=60° Balling.
2. VENTRE (3), p. 25 : at least 36° Bé.
3. OTTAVI (2), p. 268 : S.G. 1.35 (which means about 70° Brix) and 66.66% sugar by wt.
4. CRUESS (1), p. 409 : 70° Balling.

In my own investigations I noticed a spontaneous fermentation in moskonfyt of as much as 69.75° Brix, the S.G. at $\frac{17.5°C.}{17.5°C.}$ being 1.3494 by pycnometer. I therefore recommend boiling the syrup to 71° Brix, when S.G. at 17.5°C. will be 1.3572, and a gallon of this syrup at this temperature will weigh 13 lbs. 9 oz. At 100°C. or 212°F. the S.G. will be about 1.3065 and 1 gallon syrup will weigh about 13 lbs. 1 oz.

At the outset of my investigations I expected to find a concentration at which fermentation will no longer take place whilst crystallisation will not yet set in. During the course of my investigations I found a grape syrup, boiled in a vacuum pan, of 68.5° Brix and 15.30‰ total acidity, which both fermented and crystallised simultaneously. This proves that no such concentration exists as was looked for. The problem now reduces itself to finding out how moskonfyt must be prepared to have it at 71° Brix without running the risk of any subsequent crystallisation. My investigations of this problem will be continued during 1923.

A Preliminary Report on the Intracellular Symbionts of South African Coccidae.*

BY

CHAS. K. BRAIN, D.Sc., M.A., Professor of Entomology, University of Stellenbosch, South Africa.

GENERAL CONSIDERATIONS OF THE SUBJECT.

The Insects are usually looked upon as the most successful class of animals existent at the present time, and this success is mainly attributed to their mode of life.

The eggs are laid on, or near to, the food of the larvae and the young devote their time exclusively to the assimilation of food and growth. When the maximum size and weight have been reached the change to the pupal stage occurs, and the development of the adult-organs proceeds, the mature insect emerging therefrom, often within a few days. Adult insects usually require little or no food; in fact some, such as the males of Coccids, are even without mouthparts.

The span of adult life is devoted entirely to reproduction and is in many cases extremely short, sometimes lasting a few days only. During this very brief period the eggs, often in large numbers, are developed, fertilised—except the parthenogenetic females—, and deposited. In this process of development of the ova the abundant surplus supply of nutritive (fat-body) tissue accumulated during the larval period, is utilised.

This strict division of their life-cycle into distinct periods of (a) growth, (b) development, and (c) reproduction, accounts, in a great measure, for the rapidity with which insects multiply, and so for their success as a class.

* This article represents a small part of a thesis which was presented to the Faculty of Science, Birmingham University, in 1919 for the Degree of Doctor of Science. It was hoped to add to the biological facts contained herein, but lack of suitable laboratory accommodation since 1919 has prevented this from being done. I publish in this form at present, including all the insect symbionts known by me up to May, 1919, chiefly to record new species and also as a working introduction for some of my students who are ready to assist in continuing the work.

C. K. B.

Without enlarging upon the multitude of adaptations to environment exhibited in insect life, it is proposed merely to raise the question whether there may not be another very important, but yet unrecognised factor in the rapidity of production of the numerous ova in many classes of insects.

Although well known to a few observers, it is not generally recognised that there exists a true symbiosis between many insects and a number of various microscopic organisms of the *Saccharomyces* or somewhat similar type. These may be found free in the haemolymph, enclosed in the connective fat-tissue, or inhabiting a special tissue known as the "mycetom." In all instances, however, they continue to multiply at the expense of, but without apparent injury to, the host insect, until the ova are being developed, when the symbiont organism, in whatever form it may be present, makes its way into the developing ova. So general is this process that every ovum has its quota of symbiotic organisms within it at the time it enters the oviduct. In one case, that of *Coccidomyces dactylopii* (Buchner) (Plate XI) it has been established that a mass of the organisms plays an important rôle in the development of the embryo. The benefit of such an association to the micro-organism is apparent, with the protection from external influences and an abundance of food, but the only references to any advantage which may accrue to the host insects found in the literature which has yet appeared on the subject are as follows:—

Vejdovsky in 1906, in dealing with *Kermincola kermesina* Sulc, suggests that after the ova have been developed the symbionts consume the remains of the body contents of the mother insect and convert her integument into a shield to protect the progeny. It is obvious that this hypothesis, which might apply to most of the Coccids, has no significance in the case of the Psyllids, Aleurodes, Cicadas and oviparous Aphides, where the eggs are laid and left, usually without covering of any kind.

Sulc (38) deals with the problem at some length and indicates three possibilities.

(a) The host insect may benefit from enzyme action produced by the symbiont organism in that this may assist in the elaboration of the sugars contained in the sap of plants which constitutes the food of the Homoptera.

(b) Symbionts may assist in metabolism and function in the decomposition of urea as suggested by the interaction of Cyclostoma and certain bacteria. This might obtain in the aphides

and coccids where the Malpighian tubes are rudimentary or absent, but not in cicadas, etc., where these organs are well developed.

(c) Many symbionts indicate at least close relationship with yeasts, and, since these latter have been used for a long time in medicine and many species have a proved bactericidal effect, they may protect the host insect tissues from bacterial infection. As an actual example the finding of Mercier in 1906 is recorded as follows :—

> "Dieser Hefe-Bakterien Antagonismus wurde neuerdings durch Mercier (06) auch auf den Schnitten festgestellt, bei Gelegenheit des Studiums über die Bakteroiden der Blattiden; Verfasser hat gefunden, dass die symbiotischen Schabenbazillen (*B. Cuénotti*) sofort aus den von ihnen prallgefüllten Fettzellen weichen, wenn die Hefe (L'organisme à forme levure parasite de la Blatte) dortselbst erscheint: 'En effet, les Bacilles disparaissent chez les Blattes parasitées, au fur et à mesure que progresse l'envahissement du tissu adipeux par la levure.'"

It must be admitted that, with our present knowledge, it is impossible to show with certainty any precise advantage—morphological, or bio-chemical—arising from this symbiotic relationship beyond, of course, the obvious benefit to the micro-organisms.

I repeat my former suggestion that these organisms are symbionts in nature as well as in name, in that they probably perform the enzymic function of assisting to break down the stored up fat-body, reducing it to such a form that it can be utilised rapidly in nourishing the developing ova. That is to say they play an important part in the metabolism of the host insect during the period of ova production.

The possibility of the mechanical fertilisation of eggs* of some of the lower animals has apparently been demonstrated, and I would suggest that since the symbiotic organisms of aphides and coccids are so minute, their invasion of the egg amounts to little more than mechanical interference and might account for some of the parthenogenetic forms found in aphides and coccids.

Numerous other possibilities suggest themselves as interesting fields for speculation and observation. Many insects of different orders cause galls, often characteristic growths of specific size and shape, on the leaves or stems of their host plants, while others, apparently identical in form and habit, have no obvious

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* See Loeb, Science xxxvi, p. 255, Aug. 1912, etc.

effect. This class comprises chiefly psyllids and coccids in the Hemiptera. Further, *Aspidiotus perniciosus* Comst. has a distinctly injurious effect on the plants it infests and *Coccus indicus* Green actually kills its host-plant (*Opuntia monocantha*) within a few months of infesting it. On the other hand many scale insects, including *Coccus cacti*, the cochineal insect of commerce, which also lives on a species of *Opuntia*, have no ill effects on their host-plants. These, together with many other similar cases, seem to preclude the possibility of mechanical cause and suggest that the effects may result from extracellular toxins liberated within the insects by intracellular organisms, possibly symbionts.

In this connection the following extract from Sharp* is of particular interest. In discussing the galls formed by the Cynipidae (Hymenoptera) he writes :—

> "A great deal of discussion has occurred relative to the nature and origin of galls, and many points still remain obscure. Considerable light has been thrown on the subject by the direct observations of modern naturalists. Previous to Malpighi, who wrote on the subject two hundred years ago, it was supposed that the galls were entirely vegetable productions, and that the maggots found in them were due to spontaneous generation, it having been an article of belief in the Middle Ages that maggots in general arose from the various organic substances in which they were found, by means of the hypothetical process called, as we have said, spontaneous generation. Malpighi was aware of the unsatisfactory nature of such a belief, and having found by observation that galls arose from the punctures of Insects, he came to the further conclusion that the growth of the gall was due to the injection by the Insect into the plant of a fluid he termed Ichor, which had, he considered, the effect of producing a swelling in the plant something in the same way as the sting of a bee or wasp produces a swelling in an animal. Réaumur also made observations on the gall-insects, and came to the conclusion that the latter part of Malpighi's views was erroneous, and that the swelling was not due to any fluid, but simply to irritation caused by the prick; this irritation being kept up by the egg that was deposited and by the subsequent development of the larva. Observations made since the time of Réaumur have shown that the matter is not quite so simple as he supposed, for though in the case of some galls the development of the gall commences immediately after the introduction of the egg, yet in other cases, as in the Cynipidae, it does not occur till some time thereafter, being delayed even until after the hatching of the egg and the commencement of the development of the larva. Galls are originated by a great variety of insects, as well as by mites, on many plants. . . . . The exact way in which a Cynipid affects the plant is perhaps not conclusively settled, and may be found to differ in the cases of different Cynipidae, but the view

* Cambridge Natural History Insects I, pp. 525, 526. 1910.

advocated by Adler and others, and recently stated by Riley,* seems satisfactory; it is to the effect that the activity of the larva probably affects the meristem, by means of a secretion exuded by the larva. The mere presence of the egg does not suffice to give rise to the gall, for the egg may be deposited months before the gall begins to form. It is for the same reason improbable that a fluid injected by the parent fly determines the gall's growth."

The symbiont mass (mycetom) of some insects, e.g. cercopids, possesses a brilliant crimson colour suggesting that the red, violet, and pink body colour of some coccids and even the cochineal colouring substance produced in a greater or less degree by all species of the genus *Coccus* and some of the *Tachardias* may be merely a product of intracellular organisms. This possibility will probably be referred to again in later publications, but it will suffice at this stage to mention that red and yellow pigments are commonly produced by chromogenic bacteria.

Certain bacteria produce violet pigments soluble in alcohol, but insoluble in water, ether, benzine and chloroform. These are coloured yellow when treated in a dry state with sulphuric acid and emerald green with potash solution. This is mentioned because a few species of *Pseudococcus*, e.g. *filamentosus* Ckll., *solitarius* Brain and *natalensis* Brain (Cocc. of S.A. I, p. 41) are deep violet coloured, but turn bright emerald green in boiling KOH, the colour remaining insoluble in the solution but is quickly removed when transferred to alcohol.

A fact of importance in pigment formation by micro-organisms which should be borne in mind in the study of intracellular symbionts, especially in attempts to grow them in artificial cultures, is that, in connection with bacteria, it has been demonstrated that *all conditions which are unfavourable to the growth of the organism decrease the production of pigment.*

Until the last few years biologists have paid very little attention to the subject of intracellular symbionts in insects. Earlier workers in insect anatomy, Leydig (1854), Huxley (1858), Metschnikoff (1877), Putnam (1880), and Berlese (1893) referred to organs or organisms of which the exact nature was unknown, and whose function was obscure. It is mainly due to the work of Buchner, Sulc, and Pierantoni that these have now been studied from a new viewpoint, and their nature and character to some extent elucidated.

For a general account of the history of the subject, Dr. Paul Buchner's "Studien" (7), which also contains an account of the

* Science (N.S.) 1, 1895, p. 457.

various organisms recorded in the different orders of insects up to that date, may be consulted.

The *Coccidae* constitute a compact, specialised group of the Hemiptera whose nearest relatives are the Aphides, Aleurodes and Psyllides. The family now comprises approximately 2,500 species described from all temperate and tropical parts of the world. In South Africa 246 species are known, but this figure only represents perhaps a half of the actual number that exist in the country. Their strict parasitic habit has resulted in extreme degeneration accompanied by specialisation along several lines. The *Monophlebinae, Ortheziinae, Pseudococcinae, Coccinae, Lecaniinae* and *Margarodinae* generally retain legs and antennae in the adult female stage; in the remainder they are more or less atrophied or absent as in the *Diaspinae*.

Parthenogenesis has been developed to a large degree, and, while males occur in large numbers in some species, there are very common forms of which the male is unknown. All known males are highly specialised and possess but two wings as in the *Diptera*, and all agree in the absence of mouthparts of any kind.

METHODS AND TECHNIQUE.

For the rapid determination of the presence of symbionts in coccids young female specimens are chosen. From these smears are made by crushing out the whole body content between clean slides. The smears are then dried rapidly in air, and fixed by passing over a spirit flame several times, or as controls, in liquid fixative. They are next stained with Loeffler's methylene blue, for about one minute, washed under a running tap, and dried between fine filter papers. The preparation can at once be examined under the oil-immersion power of the microscope. Control slides are stained with Ziehl's Carbol-Fuchsin or Ehrlich's Haematoxylin.

Young female specimens are preferred for the larger proportion of fat-body and smaller proportion of ova they contain. With a little practice it is quite an easy matter to distinguish the different insect tissues in such smears and to determine the presence of a mycetom or free-living organisms. The methylene-blue method of staining is particularly useful for the *Lecaniid* type of symbiont, as, besides being rapid and easily controlled, it has the advantage of bringing out very distinctly all division forms of the organisms.

When the presence of organisms is demonstrated by these means, fresh material is fixed, hardened, dehydrated and embedded in paraffine for future treatment and the cutting of serial sections. The most useful fixatives for this class of work seem to be Leeuwen's picric acid, chloroform and alcohol solution (Pierantoni), or Lang's corrosive acetic solution. Large specimens should be punctured with a fine needle, and in all cases differentiation through the alcohols should be very gradual. The most useful stain for sections is Heidenhain's iron-haematoxylin followed by water eosin or orange G. Pierantoni found Leeuwen's fixative and Obst's staining method particularly suitable for his studies of *Icerya purchasi*.

For growing the organisms in culture, ordinary bacteriological methods are followed, but unusual culture media have to be employed for some of the organisms. These comprise Pasteur's yeast water and cold malt extract (wort), as used in cultivating Saccharomyces and similar organisms in connection with the study of brewing, and culture media made up from insect tissues. A medium made from bee-larvae was used in the Department of Agriculture at Washington by Dr. Phillips in connection with his studies of American and European foul brood in bees, and the writer had very good results with certain symbiotic organisms on a medium made up from cockroaches, without at that time knowing of the bee larva medium used in America. These are both made in the same way as ordinary bouillon, macerated insect tissues replacing the finely chopped beef.

To obtain pure cultures of intracellular symbionts several precautions are absolutely essential, and the difficulty of the procedure is frequently increased by the smallness of the insects. Many coccids secrete a form of honey-dew and the integument is always contaminated with a small amount of this substance which is well known to be an excellent medium for the growth of "sooty" fungus (Fumago). In order to avoid gross contamination from the exterior of the body, I pass the insects quickly through a little mercuric chloride ($HgCl_2$) solution 1—400, in a watch glass and then into sterilised water which is changed several times. The insect is then transferred to a small dissecting dish, a syracuse watch glass with paraffin wax melted into the bottom, or if very small, on a clean slide in a spot of sterile water. With sterile needles the insect is teased apart under a dissecting microscope and, in the case of free living organisms

a fragment of the fat-body is taken up on a platinum needle, a tube infected, shaken up, and a plate poured. Some plates are kept in an incubator running at 37°C., others at room temperature. From this stage on the methods are those ordinarily employed in bacteriological studies.

REVIEW OF THE LITERATURE DEALING WITH THE SYMBIONTS OF INSECTS.

(Arranged in Chronological Sequence.)

The first known reference in literature to an organism which has proved to be a symbiont is to be found in Dr. Leydig's "Zur Anatomie von *Coccus hesperidum*," published in 1854. In this connection Dr. Leydig writes:—

> "Aanhangsweise will ich noch berichten, dass sich in der Leibeshöhle fast aller erwachsenen Individuen eigenthümliche Körperchen in grösster Menge fanden, die durchaus an Pseudonavicellan erinnerten. Es sind spindelförmige, scharfgezeichnete Gebilde (Fig. 5) von 0,004‴ Länge, die immer frei, nicht in Zellen eingeschlossen beobachtet werden und in Essigsäure und Natronlosung sich nicht veränderten. Ihre Vermehrungsweise liess sich aus den verschiedenen vorliegenden Formen leicht abnehmen: die eine Polspitze wächst etwas in gerader Richtung aus, dann verdickt sich disser Fortsatz zu einem rundlichen, birnförmigen Körperchen. Während dieses wächst und allmählich die Spindelgestalt des Mutterkörperchens annimmt, ändert es auch seine stelling zu letzterem dadurch, dass es mit diesem einen Winkel bildet. Hat das Tochterkörperchen die gleiche Grosse des Mutterkörperchens erreicht, so löst sich seine Verbindung mit diesem, es wird selbständig. Die bezeichnete Art der Vermehrung dürfte demnach unter den Begriff der Sprossenbildung zu stellen sein."

It will be observed that even with our present day knowledge, and improved facilities for the examination of microscopic organisms little of importance can be added to Dr. Leydig's description of the organism and its method of multiplication as seen in smears or sections. I suggest that this organism be known as *Lecaniocola parasitica* (Lindner) since it was undoubtedly that referred to in 1895 by Lindner as *Saccharomyces apiculatus parasiticus*. It was observed by Moniez and later by Messrs. Conté et Faucheron. Moniez imagined he had isolated the organism and described what he supposed was its development in culture, but his remarks and even the name he

applied to it (*Lecaniascus polymorphus*) show plainly that he was dealing with a fungus. Vejdovsky suggests that Moniez was unwittingly dealing with two separate organisms and that the fungus was probably *Alternatia tenuis*. This is almost certainly the case. Under these circumstances I consider that Moniez's name is a synonym of the fungus name and that Lindner's name must stand.

In Metschnikoff's "Untersuchungen über die Embryologie der Hemipteren," published in 1866, we find a further reference to this subject. His studies included *Aspidiotus hederae* Vall, then known as *nerii*. He observed that this species is ovoviviparous and records his observations on certain cells present in the developing ova which he considered to be homologous to the "pseudovitellus" of the Aphides. It may be mentioned that the term pseudo-vitellus was first used by Huxley in 1858 for a certain tissue found in the ovum of parthenogenetic aphides. The forms recorded by Metschnikoff, however, did not compose a definite tissue but were separate cells, at first colourless, and later becoming brown. He observed the infection of the ova and later the dissemination of the organisms throughout the tissues of the developing embryo.

A noteworthy fact is that Mark in his "Beitrage zur Anatomie und Histologie der Pflanzenläuse, inbesondere der Cocciden," published in 1877, makes no reference to the organisms previously recorded by Leydig and Metschnikoff.

In 1880 J. D. Putnam published his Biological and other Notes on *Coccidae*. In it he described, *inter alia*, the contents of the ovaries of *Pulvinaria innumerabilis* Rathv. and on pp. 326—327 remarks :—

> "*Fourth*. Small oval bodies 3 μ to 5 μ in diameter, and about 10 long, having a specific gravity greater than water (Fig. 4). When highly magnified they are seen to be composed of a greater or less quantity of fine granular matter imbedded in a rather thick coating of some transparent substance. This last, though easily stained brown by iodine, remains unaffected by eosene or magenta, except in a few instances where the external envelope appears to be imperfect, in which case the granules become stained leaving the envelope unstained. These bodies in general appear to be very uniform in size and shape, usually regularly oval, often slightly constricted in the middle. But a careful study made since the plate was etched, shows a greater variation than I before supposed. Some are seen to taper to a point at one end (Fig. 4.c.), others while preserving the oval form have a small projection at one end, in others the projection is a little larger, in others it is still larger and of an oval form, in others a similar oval body to the original, and

finally two, three or more full-sized bodies may be seen strung together end to end. In some, as Fig. 4.b, the granules appear separated into two groups. A comparatively small number of these bodies are already found in the females immediately after copulation with the males, so they may have been present before that event had taken place. They become exceedingly abundant during the final development of the eggs in the spring, at which time they are found in great numbers in all parts of the ovaries. At a certain period in the development of the egg, just before it takes final leave of the egg follicle, several of these bodies appear to enter at the head end, where they become disintegrated, and soon after this, never before, the embryo begins to form. On this account I for a long time believed them to be spermatophores, the contained granules being the spermatozoa, and that they were derived from the sperm filaments of the male by a process of breaking up into parts placed end to end, which were subsequently in some unknown manner developed into these oval bodies. It is, however, very difficult to trace any such connection, and Dr. Mark informs me that *Leydig* has observed these bodies in adult females of *Lecanium* and regards them as parasitic, bearing some relation to the *Pseudo-navicellae*. At the present moment I am not inclined to object to this view."

Since this description and the figures given by Putnam are sufficient for the identification of the organism, it is proposed that the name *Lecaniocola putnami* sp.n. be applied to it.

"Sur une champignon parasite du *Lecanium hesperidorum* (*Lecaniascus polymorphus*)" by Moniez appeared in the Bulletin of the Zoological Society of France in 1887. This refers to the organism recorded by Leydig and on page 150 Moniez writes :—

"Nous avons rencontré à Lille le parasite du *Lecanium hesperidum* dans le sang de tous les individus de cette espèce que nous avons examinés, aussi bien chez les jeunes que chez les vieux; il n'a rien de commun avec le parasite du Ver à soie et n'est même pas une Microsporidie: c'est un Champignon Ascosporé comme nous l'ont démontré ses formes de reproduction. Nous ne donnerons ici qu'un résumé de nos observations.

"Le champignon de la Cochenille des Orangers, que nous appellerons Lecaniascus polymorphus, est excessivement variable d'aspect, selon les différents états de son mycélium; sa forme la plus simple est celle d'un corps ovoide, un peu allongé, mesurant de 4 à 5 μ de longueur, sous lequel il est difficile de distinguer une conidie ou une ascospore développées. On observe très fréquemment le bourgeonnement à ce stade: il est identique à celui des Levures et ne consiste pas, comme le pense Leydig, dans la formation d'un prolongement qui se renfle ensuite. Leydig a mal interprété l'apparence du mycélium, quand la conidie s'est détachée de l'espèce de pédicule qui l'y retenait. Ce pédicule, qui persiste souvent, pendant quelque temps du moins, peut être plus ou moins développé; il est quelquefois très long, tandis que, d'autres fois, le bourgeon est sessile. Assez souvent, on voit deux conidies insérées au même point, a l'un des pôles seulement du corpuscule-mère; elles se sont développées l'une après l'autre et peuvent rester longtemps fixées sur le mycélium; il peut arriver aussi qu'elles se détachment en meme temps et

restent unies entre elles pour un temps variable. D'autrefois le mycélium présente une série de renflements bien distincts, 6—8, plus ou moins, qui sont, pour nous, homologues des conidies. Ces renflements peuvent être très courts ou très longs et de forme variée; le mycélium, dans ces conditions, atteint quelquefois 50 à 60 μ de longueur. Les conidies présentent souvent une tache claire, analogue à un noyau; des taches claires semblables sont souvent multipliées dans le mycélium bien développé, elles sont souvent remplacées par une sorte d'axe de même nature."

As previously mentioned, this description undoubtedly refers to two separate organisms, the true symbiont and a fungus, probably *Alternaria tenuis*. In support of this view it may be mentioned that the writer has isolated Leydig's organism and cultivated it in pure culture for an extended period (vide Plate III). In every case fungi have appeared in the first plates poured i.e. those made from the coccid, and these have also been observed in "hanging drop" preparations.

Professor Berlese found, in both male and female (of *Pseudococcus citri* Risso), a remarkable tissue which he called the "*corpo ovale*." He was unable to determine the function of this body, but described and figured it in both sexes of the insect. Concerning it he writes in "Le Cocciniglie Italiane" I, p. 74, 1893 :—

"Questo *corpo ovale* (tavola iv, fig. 1, H; fig. 2, u; fig. 3, u; figura ii, f) è collocato in contatto della epidermide del ventre, e non sembra contornato da membrana alcuna. Quale sia il suo ufficio, e cosa rappresenti, mi è ignoto. Certo è che esiste sempre, molto più grosso nel *D. citri* dove occupa gran parte dèl centre, più ridotte nel D. longispinus. Numerose trachee, provenienti dal ramo longitudinale ventrale, che parte dall' ultimo stigma, vi penetrano, e colle tinture carminiche si colora abbondantemente, più di tutti gli altri tessuti, eccetto i glandulari. Non ho osservato che quest' organo sia in rapporto con alcuna apertura, oppure coll' intestino.

"Questo vi si appoggio per quasi tutto il suo decorso, ma non sembra avere altre relasioni. Quando il corpo nell 'adulto è pieno d'uova, kueste si infossano entro le cellule del detto corpo ovule, che in questo caso occupano i vani esistenti fra le uova stesse. E molto probabile che sis un ammasso di sostanza nutritiva, derivata dall'intestino, appure abbia rapporti, di difficile rilievo, colla secrezione della cera."

Further, in describing the male he states :—

"Corpo ovale, corrispondente a quello delle femmine, per posizione e forma, per quanto minore per dimensioni, e meglio definito per struttura, sta al ventre, fra i testicoli a diverso grado di sviluppo, questo organo anche nelle frome della serie maschile, con riduzione però evidente, quanto a dimensioni, da larva ad adulto (tav. V, fig. 2, l; fig. 7, d; fig. 9, D). In queste forme infatti, conservando pure la sua forma decisamente ovale, o meglio sferica, l'organo avvolto nella sua sottile membrana, riesce evidentemente compostondi grosse cellule poligonali per contatto, e con grosso nucleo e nucleolo.

"Le infiltrazioni di grasso, cosi abbondanti nelle femmine, sono qui più scarse, e quasi nulle, e perciò le cellule tutte, conservano bene il loro primitivo aspetto e la loro uniformità di sviluppo.

"Nei maschi, già da tempo sviluppati, il corpo ovale è pressochè a trofizzato, e perciò, a nostro credere, esaurito nella nutrizione dell'insetto, forse per la via dello intestino che si mantiene in possibile stato di attività."

For the later references to this tissue and the recognition of its true nature see Pierantoni 1910—1913.

Two papers by Dr. Paul Lindner of Berlin are of interest in connection with *Lecaniocola parasitica* (Lindner). These are :—

(a) Saccharomyces apiculatus parasiticus in Centrbl. Bakteriol. Abt. 2, 1895.

(b) Das Vorkommen der parasitischen Apiculatus-Hefe in auf Epheu schmarotzenden Schildläusen und dessen mutmassliche Bedeutung für die Vertilgung der Nonnentaupe in Wochenschr. Brauerei, 1907.

The organisms described are stated to be from *Aspidiotus nerii* (=*hederae*), but the figures, etc., clearly indicate that this is not the case and refer to *Lecanium hesperidum* Linn. The organisms undoubtedly are those recorded by Leydig, and are named by Lindner *Saccharomyces apiculatus* var. *parasiticus*. This is of interest because Lindner is a yeast technologist of standing.

In 1914, when working on this group, the writer corresponded with Dr. Klocker of Copenhagen, who made a special study of 17 forms of *S. apiculatus*. He stated that the organism described by Lindner was certainly not *S. apiculatus* in any form. As far as can be determined up to the present, endorspores are not produced at any stage and the yeast-like organism is one of a class of true symbionts of the *Lecaniid* and possibly other groups of coccids, hence the generic name suggested, *Lecaniocola*.

The next reference is in 1901 by Konigsberger and Zimmerman (in Mededeel uit Stands Plantentuin, vol. xliv) from Batavia. Unfortunately I have not been able to see this paper, but, according to Büchner, the symbiont organism of *Lecanium viride* Green is referred to.

Dr. Berlese, in Redia (vol. iii) 1905, describes an organism he found in large numbers in the lymph of *Ceroplastes rusci* Linn. He estimated that the average number found in each insect was probably about sixty to seventy thousand. The individual organisms were mostly 6 to 7 μ long and 2 to 2.5 broad,

elongate, egg-shaped or pointed at the ends. They multiplied by budding. These are obviously of the ordinary *Lecaniocola* form, which is the general type in the *Lecaniinae* but Dr. Berlese considers that in gelatine culture media they produce mycelia and conidia. For this reason he placed it in the genus *Oospora*, and named the species *saccardiana*. It appears highly probable, however, that, as in the case of *Lecaniascus polymorphus* Moniez, two separate organisms were confused, and that the mycelium-forming type was a fungus infection. There is no doubt, however, that the *Saccharomyces* type found free in the lymph is a symbiont of the usual type, and, for the present, this will be separated and known as *Lecaniocola saccardiana* (Berlese).

The first reference to the coccid genera *Kermes* and *Physokermes* is that of Dr. Karel Sulc (S. Böhm, Ges. Wiss. Prag) in 1906. The title of the paper reads "Kermincola kermesina n.g.s.sp. und Physokermina n.sp., neue Mikroendosymbiontiker der Cocciden," and the two forms are described and figured without any reference to previous literature on the subject. The descriptions of these forms are given in the systematic part under the names *Kermincola* and *Physokermincola*, and Dr. Sulc's figures are reproduced on Plate VII, Figs. 1 and 2.

Later in the same year (1906) Vejdovsky refers to Sulc's discovery in the same publication, and after giving further particulars of the form found in *Kermes quercus*, suggests that the purpose served by these organisms is that after the ova have been developed the fungi consume the substance of the mother insect and thus convert her body into a shield to protect her progeny.

Vejdovsky also records that Stehlik found large numbers of a smaller organism in *Pulvinaria ribesiae*, but that the particulars were then unpublished.

The following interesting account of organisms of the *Lecaniocola* type appeared in Comptes Rendus cxlv, pp. 1223—1225, 1907. It is of particular importance because it is the first record of such organisms having been grown in pure culture *without* the formation of mycelia and presents the first discussion on their biological connection with the host insects. For this reason a free translation of the most important sections is appended.

> "In teasing apart the females of *Lecanium hemisphericum* we have discovered the presence of a large number of small ovoid bodies many of which are in the state of budding. When stained they exhibit the structure of yeasts. We have grown them in artificial culture media; bouillon gelatin, potato, carrot, prune juice, etc.; in a few days the yeast forms have grown vigorously, particu-

larly on carrot. In no case have we found sporulation. We have in our possession cultures 5 months old in which the yeasts appear to be encisted within a thick membrane with the protoplasm appearing as though it were in a morbid state. We have noticed the same character in specimens taken from dead insects. Never, however, have we found mycelia either in cultures or direct from the insects.

"The yeast we have taken from *Lecanium hemisphericum* presents the following characters: From fresh insect tissue it is more or less oval, generally pointed at one end, sometimes at both; it varies considerably in size; average length 26 μ, average width 13 μ; many show budding, generally at the pointed ends.

"When grown in artificial culture the size is considerably less, average length 8 μ, breadth 4 μ.

"The study of transverse sections of *Lecanium hemisphericum* show that the yeasts are present in large numbers in all connective tissue, which fills the general body cavity and corresponds with the fat body of other insects.

"They are always intracellular and in stained sections appear surrounded by clear spaces; they always exist in large numbers. It is not probable that the yeasts are taken into the body of the insect through the digestive tract; the coccids are sucking insects which live on the sap of plants. Infection through the integument would hardly explain the abundance of *Lecaniums* infested; they are insects which move little and are protected by stout chitinous cuticle. There remains the transmission by the eggs. The study of sections of females show eggs in different stages of development; the eggs contain yeasts placed most commonly directly below the chorion. This shows therefore that the propagation of the yeasts is assured by direct transmission from the mother to all her offspring and this explains the abundance of infected insects. What is the connection between the yeast and the insect? The first idea is that of parasitism, but after careful study this appears very doubtful for the following reasons: (1) every individual of *Lecanium hemisphericum* without exception shows the presence of yeasts; (2) the yeasts are present in extremely large numbers with reproductive forms in the fat body of the insect; (3) the reproductive activity of the *Lecanium* does not appear to be interfered with. The hypothesis of a simple commensalism should be rejected because of the intracellular position and the enormous numbers of budding yeasts found in a single individual of *Lecanium*. The hypothesis of a symbiosis has the three arguments given above in its favour. The yeasts live independently on the *Lecanium;* the latter does not appear to suffer harm from them; is it not possible that it benefits. We have shown that the yeasts inhabit the fat body. As a possible function, the elaboration of digestive enzymes may be supplied by products secreted by the yeasts. We do not insist, however, for the moment on such a hypothesis whose verification would be extremely difficult, but it is indicated from our observations.

"*Lecanium hemisphericum* is not the only coccid containing yeast forms. We have met with them always in very large numbers in *Lecanium oleae, Lecanium hesperidum, Pulvinaria floccifera*, etc. These present different aspects from those in *Lecanium hemisphericum*. The comparative study of all forms only allows us to define their affinities."

We have now to deal with the recent work of Pierantoni, Sulc and Buchner, the results of which appeared between 1909 and 1913. Pierantoni deals with the symbionts of *Dactylopius* (=*Pseudococcus*) *citri* Risso and *Icerya purchasi* Mask. in a thorough manner. The former refers to the "corpo ovale" of Berlese previously mentioned and Pierantoni determines the following facts concerning it. This "corpo ovale" (Plate X, figs. 2 and 3) of Berlese is in reality a large mycetom abundantly supplied with tracheae (fig. 4). It is composed of numerous large cells (sferules) each containing several smaller cells (sferettes) which in turn contain a number (10—15) elongate organisms (figs. 5, 6, 7). The infection of the egg takes place by a number of "sferettes" (Plate XI) which enter at the anterior pole between the nutritive cells and the developing ovum (fig. 2). Figures 3 and 5 are actual photographs from sections, showing intermediate stages between 2 and 4 and 4 and 6. In *Icerya purchasi* Mask. Pierantoni found that the mycetom is in two portions lying one on either side of the mid-gut. It is composed of large cells (mycetocytes) containing numerous spherical, bean-shaped and elongate organisms (Plate XII) (a). The infection of the egg takes place at the posterior pole (figs. 3, 4 and 5). Pierantoni was able to grow the organism in culture media (gelatin 8% plus *beet sugar* 20%) and traced the development of division forms in this growth (*f*). Figs. *b* and *c* show division forms drawn from the posterior pole of a developing ovum; those illustrated at *a* are from the mycetom. These organisms were named by Buchner in 1912 and are known as *Coccidomyces dactylopii* Buchner and *Icerymyces pierantoni* (Buchner) respectively.

Sulc briefly reviews the literature dealing with the subject up to 1910 and treats the symbiontic organisms of several new classes of insects. He suggests the name "mycetom" for the symbiont tissue and "mycetocyte" for the cells containing the organisms. He then establishes the genus *Cicadomyces* for a form which he names *C. ptyeli lineati*, see Plate IX, fig. 1. From the same insect (*Ptyelus lineatus* Linn.) he described and figured a second form which was later named by Buchner (*C. minor*, fig. 2). He then considers the symbionts of different groups and follows Lindner in placing the yeast-like forms in the genus *Saccharomyces*. He describes *S. macropsidis lanionis* from the lymph of *Macropsis lanio* Linn. *Cicadomyces aphalarae calthae* from *Aphalara calthae* L. (*Psyllidae*).

Schizosaccharomyces aphalarae calthae, also from *Aphalara calthae* (Plate IX, fig. 1).

,, *psyllae försteri* from *Psylla forsteri* Flor. (fig. 2).

,, *aphidis* from *Aphis amenticola* (fig. 5).

,, *chermetis strobilobii* from *Chermes strobilobius* (fig. 4).

,, *chermetis abietis* from *Chermes abietis* Linn (fig. 3).

In dealing with the Coccids he remarks:—

"Hier konnte ich alle unsere sinheimischen Gruppen untersuchen.

"*Orthezinae*. Weisen auffallend bakterienartige Organismen auf; bei ihrer Kleinheit, lässt sich nicht einwandfrei auch bei bedeutenden Vergrösserungen entscheiden, ob es sich um wirkliche Bakterien, oder den Bakterien ähnliche Hefen handelt; letztere Ansicht ist nicht so wunderbar und kann nicht ohne weiteres ausgeschlossen werden, wenn wir die Kleinheit der Chermespilze, die noch die Pilze der Orehezinen an Grösse übertreffen, in Betracht ziehen.

"*Coccinae*. Haben sowohl bei ♀♀ wie auch bei ♂♂, die Mycetocyten in ein selfbständiges Organ, Mycetom konzentriert; bei *Pseudococcus farinosus* de Geer habe ich in ihm auf Schnittserien runde und bohnen-förmige Pilze gefunden. Isoliert sind sie rundlich, bohnenförmig entweder einzeln oder Sprossverbande bildend, mit einen deutlichen Kern; ich nenne sie: *Saccharomyces Pseudococci farinosi* n.sp.

"*Lecaniinae*, haben zerstreute nicht konzentrierte Mycetocyten und freie Pilze in der Haemolymphe, wohin sie aus ersteren hineintraten.

"*Diaspidinae* haben freie Mycetocyten, aber keine freien Pilze in der Haemolymphe; soweit ich auf einen ♀ von *A. ostreaeformis* feststellen konnte (Schnittserien), sind die Mycetocyten unregelmässig im Köper verteilt und beherbergen runde Hefezellen. Ich glaube, dass ich diese Zellen seinerzeit (1893 in litt.) bei ♂♂ von *Mytilaspis pomorum* gesehen habe, so sie amoeboide Bewegungen ausübten und nebst der Hefe auch noch violette Pigmentkörper führten (mein Fund wurde von Baborova in Ihrem Dissertationsvortrage (02) besprochen); leider ist mir die damals verfertigte Abbildung verloren gegangen, und die heurige Saison ist zu vorgeschritten, um noch jetzt der Vervollständigung wegen die Untersuchungen wiederholen zu können. Es ist sicher, dass bei Diaspidinen zur Konzentrazion der Mycetocyten in ein kompaktes Organ, Mycetom, nicht kommt.

"Der Zusammenhang zwischen Mycetom ("Pseudovitellus") freien Hefezellen (Mycetocyten) und freien Hefepilzen in der Haemolymphe ist von meinen Vorgängern nicht erkannt worden."

In June of the same year (1910) Sulc describes two distinct symbionts from *Cicada orni* Linn. These are *Saccharomyces cicadarum* (Plate VIII, fig. 5) and *Cicadomyces cicadarum* (Plate IX, fig. 4).

Dr. Paul Buchner's studies (7) are the most comprehensive which have yet appeared, reviewing the whole subject up to 1912, and should be seen by everyone interested in the subject.

PROVISIONAL ARRANGEMENT OF THE INTRACELLUAR SYMBIONTS OF INSECTS, WITH A SHORT RECORD OF EVERY KNOWN SPECIES.

The following is an attempt to define the genera already established as in no case has a genus diagnosis been published except, of course, for *Bacillus*.

The characters have been obtained from the descriptions—such as they are—of the type species, but, as these descriptions were only made from smear or section preparations none of them refer to cultural characteristics, which I feel are very desirable.

The generic names employed up to the present are characterised by the affixes "cola" for forms living free in the haemolymph or connective fat tissue and "myces" for those inhabiting an obligatory mycetocyte or definite mycetom. As some such distinction is, at least, convenient, provisional generic names proposed follow the same plan. A few of the new genera founded, such as *Icerymyces, Chermomyces and Cissococcomyces* are deemed necessary for phylogenetic as well as morphological reasons.

Genus *Bacillus* Cohn.

Schizomycetes, Order *Eubacteria*, Family *Bacteriaceae Migula*.

The cells are cylindrical, of longer or shorter length. The rods are sometimes oval in shape. Cells are motile and possess flagella which are distributed over the entire surface. Endospore formation occurs with marked regularity. The bacteria in this genus are motile only during certain periods of their life. This period varies greatly in length and occurs only in the vegetative stage.

1. *Bacillus cuenoti* Mercier 1906.

(Plate I, Figs. 1—8.)

Specimens of the common cockroach, or "blackbat" (*Periplaneta orientalis* Linn.), were collected and examined for possible symbionts. In every specimen opened *Bacillus cuenoti* Mercier was found in large numbers. The presence of this organism was demonstrated in the adult ♂ and ♀ in the larval forms, and also in eggs taken from the body of the ♀, and in the egg-capsules. For this purpose some portions were teased up, while others were fixed, sectioned and stained.

A method for the rapid demonstration of their presence is as follows: An insect may be killed and fixed by dropping into boiling water, or nearly boiling Picro-nitric if intended for sectioning. Using a fine pointed pair of scissors an incision is made all round the ventral and extreme lateral margins of the abdomen, beginning at the caudal extremity. The ventral abdominal integument may then be pulled away, towards the head, leaving the body contents attached to the dorsal surface. It will then be seen that along the lateral margins there is a dense white fat-body (fig. 1). Insert a platinum loop into this, quite close to one of the margins, and move slightly in a longitudinal direction. Remove the loop and make a smear on a prepared slide; dry for a few moments, pass through a flame and stain. Loeffler's alkaline methylene-blue is quite suitable, and has the advantage of rapidity.

The bacillus was first described by Blochmann (Zeitschr. fur Biol. Bd. xxiv, p. i, 1887). Forbes (1892), Cuenot (1892), Henneguey (1904), and Prenant (1904) also report the organism. It was named, described and figured by Mercier in the "Archiv fur Protistenkunde" ix, pp. 345—358, 1907.

Bacillus cuenoti is not found in all the fat-body cells in *Periplaneta orientalis,* but in certain definite cells which occupy a central position in the lateral masses, and which constitute a longitudinal band of tissues near the thin lateral margin of the body. These cells are well supplied with tracheae. *B. cuenoti* is also present in the developing ova and also in the egg-capsules, so that the infection is passed from one generation to the next through the egg, and is in no manner accidental or secondary. No definite arrangement has been seen to exist in the cells, the bacilli having the appearance, rather, of being packed at random, their numbers being so great that the general cytoplasm is entirely obscured (Fig. 2).

In fresh material stained with methylene blue as mentioned above, the organisms appear as thick rods 4 to 8 μ long, with broadly rounded ends. The most common length is 5 to 6 μ. Some are straight, but many appear curved. In cockroaches which have been kept in a small vessel without food for about a week the bacilli exhibit similar characters but are smaller, ranging from 3 to 6 μ, the most common size being 3 to 4 μ (Fig. 5).

Bacillus cuenoti is Gram +, but not acid-fast.

Flagella, 6 to 8?; *peritrichiate*, stained by Bunge's modification of Loeffler's method (Fig. 6).

Spores, found commonly on potato cultures, and from bottom of bouillon tubes; elliptical, broader than bacillus, usualy at one end, giving a "drum-stick" appearance to it. Stained by Ziehl's carbol fuchsin (Fig. 7).

Culture characteristics.

(a) *Agar slope*. In 24 to 30 hours at the temperature of the laboratory, there appear small rounded colonies which are at first yellowish white, slightly raised, opaque, and glistening. After three days they become confluent, forming a yellow band with edges somewhat sinuous, and with the margin very finely striated. At a later stage the growth completely covers the surface and becomes deep amber in colour. The medium beneath is not coloured.

(a) *Potato*. On potato the growth is similar to that on agar stroke, but in cultures two weeks old (at room temperature) the colour is a little darker. It is, however, of similar gelatinous consistency.

(c) *Gelatin*. Stroke cultures on gelatin resemble those on agar after 24 hours at room temperature, but on the second day liquefaction begins at the surface and later continues throughout the mass. The liquid remains clear except for the grayish-yellow cloudy patches. In gelatin-stab cultures the growth extends the whole depth of the stab, liquefaction begins from here and gradually extends outwards.

(d) *Nutrient broth*. After 24 hours the liquid becomes slightly cloudy, cloudiness increasing up to about the 7th day. About the 3rd day a thin film appears on the surface, which remains delicate and very fragile, and flat to the edges of the flask; this gradually breaks up and sinks. In two weeks the liquid becomes clear, but is somewhat darkened, and the bacilli at the bottom in almost all cases contain spores.

Genus *Saccharomyces*.

Blastomycetes.

The chief characteristics of this genus are the round or oval shape; granular protoplasm containing solid particles and often vacuoles; the definite enclosing membrane; the reproduction asexually by budding and the formation of endospores.

The species recorded below has not been shown to produce endospores, but is left in the genus for the present in which Buchner placed it because there are so many points yet to be determined concerning it.

2. *Saccharomyces anobii* Buchner 1912.

(Plate II.)

This species was named by Dr. Buchner from the particulars given by Escherich in Biologisches Centralblatt xx, pp. 350—358, 1900. In the same publication for 1899 W. Karawaiew first called attention to the organism in his paper "Zur Anatomie und Metamorphose des Darmkanals der Larve von Anobium paniceus." Escherich followed on from this work and succeeded in isolating the yeast-like body from the tissues and also in growing it in a one per cent. solution of glucose. He also traced the organism through the pupal and adult stages, and gave figures illustrating different points of interest. I have copied the figures and collected them to form Plate II.

The mid gut is much larger in the larval stage than in the pupa or adult, the comparative sizes being given in Figs. 1 and 2. In the larva the majority of the cells are distended as shown in Fig. 3, by the large number of organisms they contain. *S. anobii* is also present in the pupal and adult stages but in fewer numbers. It would appear from the work of Escherich that their presence does not in any way affect pupation, nor does it interfere with the powers of reproduction.

Saccharomyces anobii as seen in the host cells is a more or less pear-shaped cell, usually broadly rounded at the one extremity and pointed at the other, about 4.5 μ long. Some forms show budding, the buds being either terminal or slightly to one side of the pointed extremity.

In a 1 per cent. sugar solution budding proceeds rapidly, two buds appearing occasionally from the same point. After 8 days in this solution a kind of mycelium was observed, the buds being rounded at both extremities and attached end to end (Fig. 6).

The form of nucleus appears from the description to agree with that observed in Saccharomyces by Wager, there being a large vacuole and one or more dark-staining bodies. After growth in the sugar solution for some days the vacuoles appear smaller and the cells show numbers of refractive granules.

The formation of spores has not been recorded.

It should be pointed out that it has not been definitely determined whether this organism should be considered a symbiont or not, but is apparently accepted as such by Buchner (7).

Genus *Lecaniocola* nov.

Synonymy :

Saccharomyces Lindner (in part).
Oospora Berlese (in part).
Lecaniascus Moniez (in part).

Organism not in a definite mycetom but free in haemolymph or connective fat tissue, ± spherical, pear-shaped or elongate. Often with one or both ends somewhat pointed. Multiplication by gemmation. This genus presents many similarities to *Saccharomyces*, but is separated by the fact that the organisms included are all found in symbiotic relationship to Coccids, particularly those of the sub-family *Lecaniinae*, and closely related forms. Further, in no case has it yet been determined that endospores are produced.

3. *Lecaniocola parasitica* (Lindner).

(Plate III.)

Lecaniascus polymorphus Moniez (in part).
Saccharomyces apiculatus var. *parasiticus* Lindner.

Host Insect : *Lecanium hesperidum* Linn.

Infection of ovum by few organisms which enter at the anterior pole between the nutritive cells and the developing ovum, exactly as shown for *Lecaniocola contéi* in Fig. 5 of Plate IV. Appearance in smears (methylene blue). This form has been repeatedly described, first by Leydig in 1854 (see p. 319), later by Moniez and Lindner. After 20 hours in a hanging drop of wort the average size rises to 10 μ long and 3 μ broad; the protoplasm exhibits a number of small vacuoles, and the majority of the organisms have one end pointed. They are always, in culture, less angular than the forms from *L.* (*Saissetia*) *hemisphaerica* Targ., mentioned later under the name *L. contéi* sp.n.

4. *Lecaniocola putnami* sp.n.

Host Insect : *Pulvinaria innumerabilis* Rathvon.

This name is applied in the form described and figured by Putnam, see p. 10.

5. *Lecaniocola contéi* sp.n.

(Plate IV.)

Host Insect: *Lecanium* (*Saissetia*) *hemisphaerica* Targ.

The organism described by Conté and Faucheron, pp. 13 and 14, remains unnamed, and I suggest that the name of the senior author be applied to it. It was grown in culture, but its cultural characteristics have not yet been thoroughly investigated. I have figured some important details on Plate IV, including photographs of the mode of infection of the ovum. A detailed comparison of this species with *L. parasitica* Lindner is being worked out and will be published later.

6. *Lecaniocola rosae* (Buchner).

(Plate VII, Fig. 3.)

Coccidomyces rosae Buchner 1912.

Host Insect: *Lecanium corni* Bché.

Organism elongate, generally with one end broadly rounded and the other pointed, sometimes with both ends pointed. Protoplasm with diffuse granules. Budding at the pointed end. Length about 8.5 μ.

7. *Lecaniocola filippiae* sp.n.

(Plate V, Fig. 1.)

Host Insect: *Filippia chilianthi* Brain.

Organism varying greatly in size and form, sometimes almost spherical, pear-shaped, lemon-shaped, or elongate. Division forms, often with several buds in a chain, may attain 25 to 30 μ in length. Stained with methylene blue the protoplasm appears finely granular, seldom with vacuoles. Average size of vegetative forms 6 to 8 μ long and 3 μ broad.

8. *Lecaniocola inglisiae* sp.n.

(Plate V, Fig. 2.)

Host Insect: *Inglisia geranii* Brain.

Organisms elongate, average length 11 μ, often parallel-sided, similar to the genus *Kermincola* Sulc. Protoplasm coarsely granular, often with a few large granules and one or more large vacuoles. Buds terminal, often remaining attached by a long "neck" until almost as large as mother cells.

9. *Lecaniocola pulvinariae* sp.n.

(Plate VI, Fig. 2.)

HOST INSECT: *Pulvinaria mesembryanthemi* Sign.

Organisms small, usually 4 or 5 μ long, pear-shaped. Protoplasm homogeneous, without granules or vacuoles (methylene blue). Buds form at the pointed end, two or three often found in a chain.

10. *Lecaniocola protopulvinariae* sp.n.

(Plate VI, Fig. 3.)

HOST INSECT: *Protopulvinaria pyriformis* Ckll.

Organisms most commonly spindle-shaped, 6 to 11 μ long, with finely granular protoplasm. Buds terminal, usually attached by a very thin, tapering neck.

11. *Lecaniocola proteae* sp.n.

HOST INSECT: *Lecanium proteae* Brain.

Organisms small, 4 to 5 μ long, and almost as broad, almost spherical with one end attenuate; protoplasm with usually one small vacuole and one large granule. Buds terminal, persistent until full grown. There appear to be a second organism present in the form of short, deeply staining rods. This may represent a disymbiotic condition in which both forms live free in the haemolymph or connective fat-tissue and will be reported upon later.

12. *Lecaniocola lecanii viridi* sp.n.

HOST INSECT: *Lecanium viride* Green.

This name is proposed for the forms referred to by Konigsberger and Zimmermann in 1901.

13. *Lecaniocola egbarum fulleri* sp.n.

HOST INSECT: *Ceroplastes egbarum fulleri* Ckll.

Organism similar to *L. ceroplastidis pallidi* but broader with coarser protoplasm. Buds similar but attached by longer and thinner "necks."

14. *Lecaniocola saccardiana* (Berlese) 1906.

Oospora saccardiana Berlese (in part) (see p. 13).

HOST INSECT: *Ceroplastes rusci* Linn.

Berlese, as already recorded, described and figured this species, but in all probability he was dealing with a mixed infection, the symbiont and a fungus. The cultural characteristics are therefore, for the present, disregarded.

Organism not forming a mycelium in the host-insect; free, Saccharomyces-like in form; long egg-shaped, often with both ends pointed and then lemon-shaped; mostly 6 to 7 μ long and 2 to 2.5 μ broad, with granular protoplasm. Budding mostly from one end, occasionally at both.

15. *Lecaniocola ceroplastidis pallidi* sp.n.

HOST INSECT: *Ceroplastes pallidus* Brain.

Organism of the usual type, generally with one end narrow or pointed, about 6 to 8 μ long. Protoplasm coarse with one or two large granules and often a few vacuoles. Buds persistent, sometimes three together in a chain.

16. *Lecaniocola*(?) *macropsidis lanionis* (Sulc.) 1910.

Saccharomyces macropsidis lanionis Sulc.

HOST INSECT: *Macropsis lanio* Linn. (Jassidae).

Organism elongate egg-shaped or with one or both ends pointed; 3 μ long and 1 μ broad. Protoplasm coarse; nucleus distinct, round, generally central. Budding terminal; buds at first elliptical, then egg-shaped with the broad end towards the mother-cell; vacuolate, never in chains.

17. *Lencaniocola*(?) *conomeli limbati* (Sulc.) 1910.

Saccharomyces conomeli limbati Sulc.

HOST INSECT: *Conomelus limbatus* Fabr. (Fulgoridae).

Organism mostly elliptical or egg-shaped; nucleus small; protoplasm coarse. Buds terminal or slightly lateral; at first round, then egg-shaped, not separating until as large as mother-cells. Never in chains.

Genus *Kermincola Sulc.*

Organism not in a definite mycetom, very elongate (about 20 μ), narrow, with parallel sides; one extremity usually pointed. Nucleus very large and distinct, generally towards the pointed end. Some individuals extremely long (40 to 50 μ) with two to four nuclei. Outer membrane dense, hyaline. Multiplication by budding.

Type *K. kermesina* Sulc. in *Kermes quercus* Linn.

18. *Kermincola kermesina* Sulc 1906.

(Plate VII, Fig. 1.)

Organisms mostly elongate, 20 μ long and 4 μ broad, with parallel sides, and often with one or both ends pointed.

Nucleus very large and distinct; protoplasm granular; periphery hyaline. Occasionally very long (40 to 60 μ) specimens are seen with 2 to 4 nuclei. Budding terminal.

19. *Kermincola tenuis* (Buchner).

Psyllidomyces tenuis Buchner 1912.

Organism similar to *K. kermesina* in many respects; living in fat cells and haemolymph of a Psyllid.

This is described and figured by Buchner 1912 (Taf. 5, figs. 4 and 7).

Genus *Physokermincola* nov.

Organism not in a definite mycetom, pear-shaped or spindle-form, about 10 μ long, with one or both ends sharply pointed. Outer membrane not dense; nucleus exceptionally large; multiplication by budding.

Type *P. physokermina* (Sulc.) in *Physokermes abietis*.

20. *Physokermincola physokermina* (Sulc.) 1906.

Kermincola physokermina Sulc. 1906.

Organism about 10 μ long and 3 μ broad, pear-shaped or pointed at both ends and then spindle-form. Protoplasm dense, without hyaline periphery; nucleus very large. Budding terminal.

Genus *Cicadocola* nov.

Organism not in a definite mycetom, very elongate, 10 μ or more, narrow, usually with one extremity attenuated but not sharply pointed. Outer membrane not dense; one or more nuclei present but small. Multiplication by budding; buds often persistent, often four or five united in a long chain.

Type *C. cicadarum* (Sulc.) in *Cicada orni*.

21. *Cicadocola cicadarum* (Sulc.) 1910.

(Plate VII, Fig. 5.)

Saccharomyces cicadarum Sulc.

Organism living in the connective fat-tissue or haemolymph of *Cicada orni*, elongate, 10 to 12 μ long and 2 to 3 μ broad. Protoplasm finely reticulate; one or more small nuclei often visible, generally near the broad end; a single vacuole commonly present. Buds terminal, persistent, often 4 or more united in a chain.

Genus *Cicadomyces* Sulc.

Organisms enclosed in a definite mycetom, spherical, bean-shaped or roundly polygonal, 6 to 10 μ in diameter; cytoplasm with large vacuoles and several large granules. Multiplication by both budding and fission; budding forms often in long chain-like groups.

Type *C. ptyeli lineati* Sulc. in *Ptyelus lineatus*.

22. *Cicadomyces ptyeli lineati* Sulc. 1910.

(Plate IX, Fig. 1.)

(=form 1 from Sulc.)

Organisms living in the large carmine coloured mycetom of *Ptyelus lineatus;* 6 to 10 μ long, spherical, bean-shaped or roundly polygonal. Protoplasm granular with large vacuoles. Multiplication by budding and fission; often united into long chains.

23. *Cicadomyces minor* Buchner.

(Plate IX, Fig. 2.)

=*C. ptyeli lineati* form II of Sulc.

This organism is found in the small yellow mycetom of *Ptyelus lineatus*. The individual cells are much smaller than the above-mentioned species, averaging 3 μ in diameter. They are, moreover, distinct in form, Cf. Figs. 1 and 2, Plate IX.

24. *Cicadomyces aphalarae calthae* Sulc. 1910.

(Plate IX, Fig. 3.)

Organisms enclosed in a definite mycetom of *Aphalara calthae* (Psyllid). Similar to *C. ptyeli lineati* but more spindle-shaped (Fig. 3). This is form I of Sulc.)

25. *Cicadomyces cicadarum* Sulc.

(Plate IX, Fig. 4.)

Organisms very variable in form (Fig. 4) but always with coloured granules; always in a definite mycetom.

26. *Cicadomyces liberiae* Buchner.

Organisms inhabiting the peripheral portion of the mycetom of a cicada from Liberia. Organisms usually round, oval, or elongate, more nearly resembling the forms of *Cissococcomyces*.

27. *Cicadomyces minimus* Buchner.

Organisms inhabiting the inner portion of the mycetom of the cicada mentioned in the previous note. Individual organisms very small, from 1 to 3 μ in diameter.

28. *Cicadomyces dubius* Buchner 1912.

This form has never been described at all but is figured in Buchner's Taf. 5, Fig. 8, 1912. It is found in the central portion of the mycetom of a Psyllid.

29. *Cicadomyces aphrophorae alni* Sulc. 1910.

This is another undescribed form found by Sulc in the mycetom of *Aphrophora alni*.

30. *Cicadomyces aphrophorae salicis* Sulc.

HOST INSECT: *Aphrophora salicis*.

Organisms living in inner portion of mycetom, elongate, parallel-sided, often curved.

31. *Cicadomyces rubricinctus* Buchner 1913.

HOST INSECT: *Aphrophora salicis*.

Organisms found in the second mycetom of host insect, i.e. the orange red portion reported by Sulc. Similar to form mentioned above (30) but thicker.

Genus *Cissococcomyces* nov.

(Plate VI, Fig. 1, b and c.)

Organisms enclosed in obligatory mycetocytes resembling large oenocyte cells (b); spherical, oval, or elongate, closely resembing some of the described species of *Cicadomyces*, e.g. *C. Liberiae* Buchner, *C. minnimus* Buchner, etc.

Type *C. natalensis* sp.n. from *Cissococcus fulleri* Ckll.

32. *Cissococcomyces natalensis* sp.n.

(Plate VI, Fig. 1, b and c.)

Organisms very variable in form and size; uniformly deep staining, Fig. 1, b and c.

Genus *Coccidomyces* Buchner.

(Plates X and XI.)

Cells of the mycetom, i.e. myctocytes or "sferules" invaded by smaller cells (sferettes) each containing ten to fifteen organisms which multiply until the cytoplasm of the larger cell is obscured and the nucleus and cell remnants are crowded to (usually) the centre of the cell.

Individual organisms small, about 2 to 4 μ long, round, oval, elongate or sickle-shaped. Infection of the ovum by a number of "sferettes" containing numerous organisms.

Type *C. dactylopii* Buchner in *Pseudococcus citri* Risso.

33. *Coccidomyces dactylopii* Buchner.

(Plates X and XI.)

"Corpo ovale" Berlese, see p. 11.

The chief characters are well shown in Plates X and XI and will be dealt with in detail in a future report.

Genus *Icerymyces* nov.

(Plate XII.)

Organisms in a definite mycetom, round, oval, or pear-shaped, always with a distinct outer membrane. Multiplication by fission, the cytoplasm completely dividing before the outer membrane constricts. Infection of the ovum by single organisms, not enclosed in a mycetocyte as in *Coccidomyces*.

Type *I. pierantonii* Buchner in *Icerya purchasi* Mask.

34. *Icerymyces pierantonii* (Buchner).

(Plate XII.)

Coccidomyces pierantonii Buchner 1912.

The various forms of this organism have been described and figured in the excellent papers by Dr. Pierantoni as recorded on p. 15. Further cultural details will be given later in a comparative study of forms found in the *Monophlebinae*.

Genus *Aleurodomyces* Buchner.

Organisms in a definite mycetom, round or oval, with a pear-shaped vegetative form. Cytoplasm coarse, often with large vacuoles, and, in the vegetative form often with a colouring substance. Nucleus small. Multiplication by budding. Mycetocytes 8 to 13 μ in diameter; individual organisms 2 to 5 μ in diameter.

Type *A. signoretii* Buchner, in *Aleurodes* sp.

35. *Aleurodomyces signoretii* Buchner.

(Plate VII, Fig. 4.)

Organisms enclosed in obligatory mycetocytes of *Aleurodes* sp; round, oval, or deformed by pressing one against another. This species is figured in Buchner's Taf. 4, 1912.

Genus *Aphidomyces* nov.

=*Schizosaccharomyces* Buchner.

This genus is established for those small forms of $\pm$ spherical organisms which are found as symbionts in the Aphides and related insects and these constitute the "pseudovitellus." They

resemble *Schizosaccharomyces* in many respects, especially in their mode of multiplication, i.e. by fission. A few have been recorded in which spore-formation is known.

36. *Aphidomyces aphalarae calthae* (Sulc.).

(Plate VIII, Fig. 1.)

Saccharomyces aphalarae calthae Sulc 1910 and Buchner (1912).

This organism is found in association with two other fungi in the mycetom of *Aphalara calthae* (Psyllid), Fig. 1.

Spore formation was observed in this species by Sulc, who states that generally 3 are formed in each cell.

37. *Aphidomyces aphidis* (Sulc.) 1910.

(Plate VIII, Fig. 5.)

Saccharomyces aphidis Sulc. 1910 and Buchner 1912.

Organisms ± spherical or nearly so, about 4 μ in diameter, living in the obligatory mycetocytes of *Aphis amenticola*. Multiplication by fission. Spore formation as in the previous form.

38. *Aphidomyces drepanosiphi* (Buchner).

(Plate VIII, Fig. 6.)

This is the form described and figured by Buchner in 1912 (Taf. 1) from *Drepanosiphum* sp.

39. *Aphidomyces psyllae försteri* (Sulc) 1910.

(Plate VIII, Fig. 2.)

Schizosaccharomyces psyllae försteri Sulc 1910 and Buchner 1912.

This is a distinct form living in a mycetom in *Psylla fösteri*. The cells are oval or egg-shaped instead of spherical.

40. *Aphidomyces sulcii* (Buchner) 1911.

Cicadomyces sulcii Buchner 1911.

Schizosaccharomyces sulcii Buchner 1912.

This is a very distinct form described and figured by Buchner from the fat-body of a Japanese cicada. It possesses several striking and unusual characteristics and does not appear to belong, properly, to any of the known genera. I allow it to stand, for the present, in the hope of discovering other forms showing similar characters.

Genus *Chermomyces* nov.

Organisms in a definite mycetom; as in *Aphidomyces* but elongate, often with ends ± pointed. Cytoplasm vacuolate, nucleus distinct. Multiplication by transverse fission.

Type *C. chermetis strobilobii* Sulc. in *Chermes strobilobius*.

41. *Chermomyces chermetis strobilobii* Sulc.

(Plate VIII, Fig. 4.)

Schizosaccharomyces chermetis strobilobii Sulc 1910 and Buchner 1912.

Organisms living in a definite mycetom in *Chermes strobilobius* Kalt. 1 to 2 μ long, elongate (Fig. 4). Division by transverse fission.

42. *Charmomyces chermetis abietes* Sulc.

(Plate VIII, Fig. 3.)

Schizosaccharomyces chermetis abietes Sulc. 1910 and Buchner 1912.

Organisms living in the mycetom of *Chermes abietes* Linn.; long, oval, broader and more parallel sided than previous species. Cf. Figs. 3 and 4, Plate VIII.

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EXPLANATION OF PLATES.

Plate I. (Figs. 3—8 after Mercier.)

1. Adult female *Periplaneta orientalis*, showing position of fat-body (original).
2. Cell packed with *B. cuenoti* Mercier (original).
3. *B. cuenoti* from 15-hour bouillon culture at 30° C.
4. *B. cuenoti* from tissue of well-nourished insect.
5. *B. cuenoti* from tissue of insect kept without food for seven days.
6. *B. cuenoti*, 12-hour culture on gelatine showing flagella.
7. *B. cuenoti* spores (on potato), stained Ziehl's Carbol Fuchsin —Methelene-blue.
8. Portion of a transverse section of egg of *P. orientalis* fixed in Zenker's Fluid, stained Iron Haematoxylin.

Plate II. (After Escherich.)

1. Transverse section through a larva of *Anobium paniceum*.
2. Transverse section through the mid-gut of pupa, same magnification.
3. Portion of section through the mid-gut of adult insect.
4. Different forms of the fungi from the gut-cells.
5. Different forms of the fungi after 2 days' culture in 1% grape sugar.
6. Different forms of the fungi after 8 days' culture in 1% grape sugar.

Plate III (original).

1. *Lecanium hesperidum* Linn. on leaf.
2. *Lecaniocola parasiticus* (Lindner). After 24 hours in yeast water.
3. *Lecaniocola parasiticus* (Lindner). After 55 hours in yeast water.
4. *Lecaniocola parasiticus* (Lindner). Forms from insect tissue.
5. *Lecaniocola parasiticus* (Lindner). Distribution in insect tissue.
6. *Lecaniocola parasiticus* (Lindner). Four days' growth in wort, at room temperature.

PLATE IV (original).

1. *Lecanium* (*Saissetia*) *hemisphaerica* Targ. on stem.
2. Transverse section, fixed Lang's Corrosive Acetic Stained Iron-haematoxylin, showing *Lecaniocola contei* in connective tissue.
3. *Lecaniocola contei* sp.n. in situ, further enlarged.
4. *Lecaniocola contei* after 55 hours in yeast water.
5. *Lecaniocola contei* infecting ovum of *L. hemisphaerica* Targ.
6. *Lecaniocola contei* 4 days' growth in wort, at room temperature.

PLATE V (original).

1. *Filippia chilanthi* Brain on stems of host-plant (photograph) and *Lecaniocola filippiae* sp.n. from connective fat-tissue of same.
2. *Inglisia geranii* Brain (photograph) and *Lecaniocola inglisiae* sp.n. from connective fat-tissue of same.

PLATE VI (original).

1. Gall and insect exposed of *Cissococcus fulleri* Ckll. (photograph). *a*, Normal fat-cell (œnocyte?); *b* and *c*, fat-cells ± filled by *Cissococcomyces natalensis* sp.n.; *d*, *Lecaniocola cissococci* sp.n. in connective fat-tissue; and *e*, bacteria? from connective fat tissue of same insect.
2. *Pulvinaria mesembryanthemi* Sign. on leaves (photograph) and *Lecaniocola pulvinariae* sp.n. from connective fat-tissue of same.
3. *Protopulvinaria pyriformis* Ckll. on leaf (photograph) and *Lecaniocola protopulvinariae* sp.n. from connective fat-tissue of same.

PLATE VII. (After Buchner and Sulc.)

1. *Kermincola kermesina* Sulc. from connective fat-tissue of *Kermes quercus*.
2. *Physokermincola physokermina* (Sulc) from connective fat-tissue of *Physokermes abietis*.

3. *Lecaniocola rosae* (Buchner) from connective fat-tissue of *Lecanium corni* Bche.
4. *Aleurodomyces signoretii* Buchner from mycetocytes of *Aleurodes* sp.
5. *Cicadocola cicadarium* (Sulc) from connective fat-tissue of *Cicada orni*.

PLATE VIII. (After Sulc, Buchner and Huxley.)

1. *Aphidomyces aphalarae calthae* (Sulc).
2. *Aphidomyces psyllae forsteri* (Sulc).
3. *Chermomyces chermetis abietis* (Sulc).
4. *Chermomyces chermetis strobilobii* (Sulc).
5. *Aphidomyces aphidis* (Sulc).
6. *Aphidomyces drepanosiphi* (Buchner).

7 and 8. Copies of Huxley's original figures from Trans. Lin. Soc. xxii, the origin of the term "Pseudovitellus."

PLATE IX. (After Sulc and Buchner.)

1. *Cicadomyces ptyeli lineata* Sulc.
2. ,, *minor* Buchner.
3. ,, *aphalarae calthae* Sulc.
4. ,, *cicadarum* Sulc.

PLATE X. (Figs. 4—7 after Pierantoni.)

1. *Pseudococcus citri* (Risso) x 25 (original).
2. ,, ,, section showing large, ventral mycetom (original).
3. ,, ,, Berlese's figure showing large "corpo ovale."
4. ,, ,, Portion of mycetom showing tracheae.
5. Portion of mycetom showing Pierantoni's "sferules" and "sferettes."
6. Sferettes enlarged still more showing organisms (*Coccidomyces dactylopii* Buch).
7. Different forms of *Coccidomyces dactylopii* Buchner.

Plate XI.

1. Eggs of *Pseudococcus citri* (Risso) enclosed in waxy covering (original).
2. Pierantoni's figure of mode of infecting the ovum (*Coccidomyces dactylopii*).
3. Photograph of slightly later stage (original).
4. Pierantoni's figure of later stage.
5. Photograph of almost identical stage (original).
6. Pierantoni's figure of completed "polar mass."

Plate XII. (After Pierantoni, except Fig. 1.)

1. *Icerya purchasi Mask* (original).
2. Pierantoni's figure of portion of the abdomen of *Icerya purchasi* showing position of mycetom.
3. Pierantoni's figure of portion of the oviduct with symbionts entering.

4 and 5. Later stages showing entrance of symbionts into the developing ovum.

a. *Iceymyces pierantonii* (Buchner) from mycetom.
b. ,, ,, stained with carbol-fuchsin
c. ,, ,, from the body cavity.
d. Other forms from the body cavity.
e. Other from posterior pole of developing ovum.
f. Forms found in culture (sugar-gelatine).

PLATE I.

Chas. K. Brain.

PLATE II.

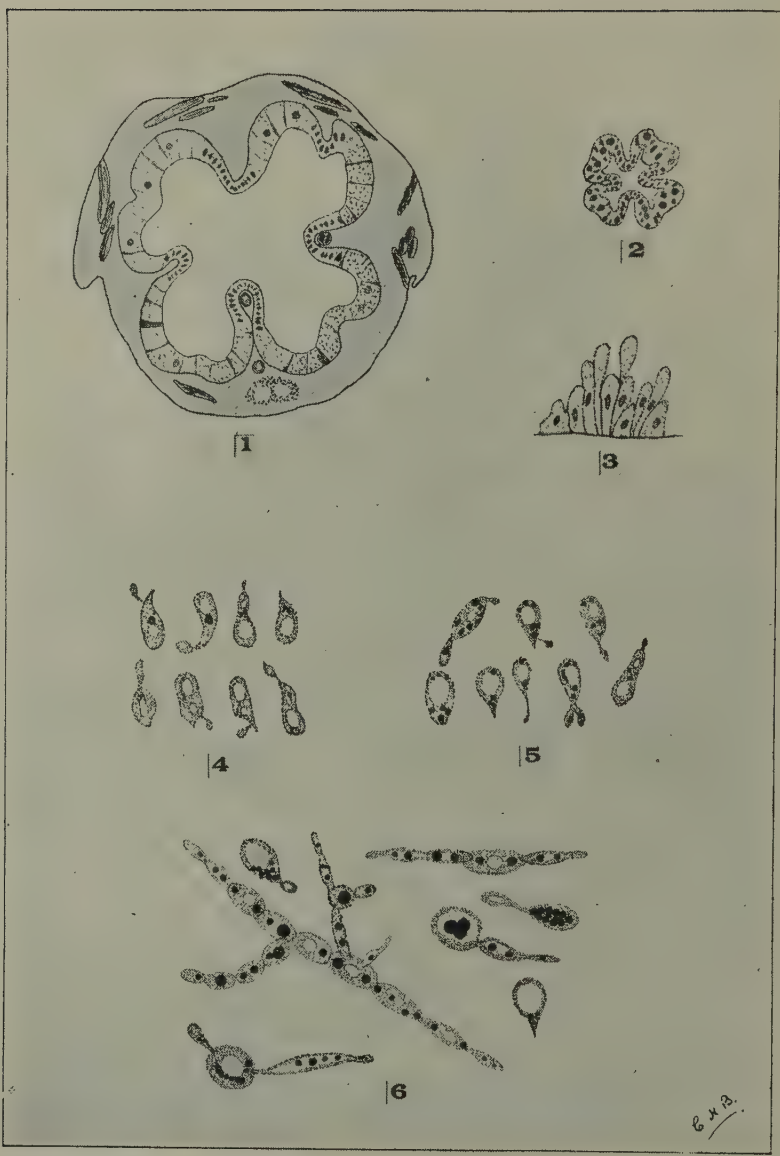

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PLATE III.

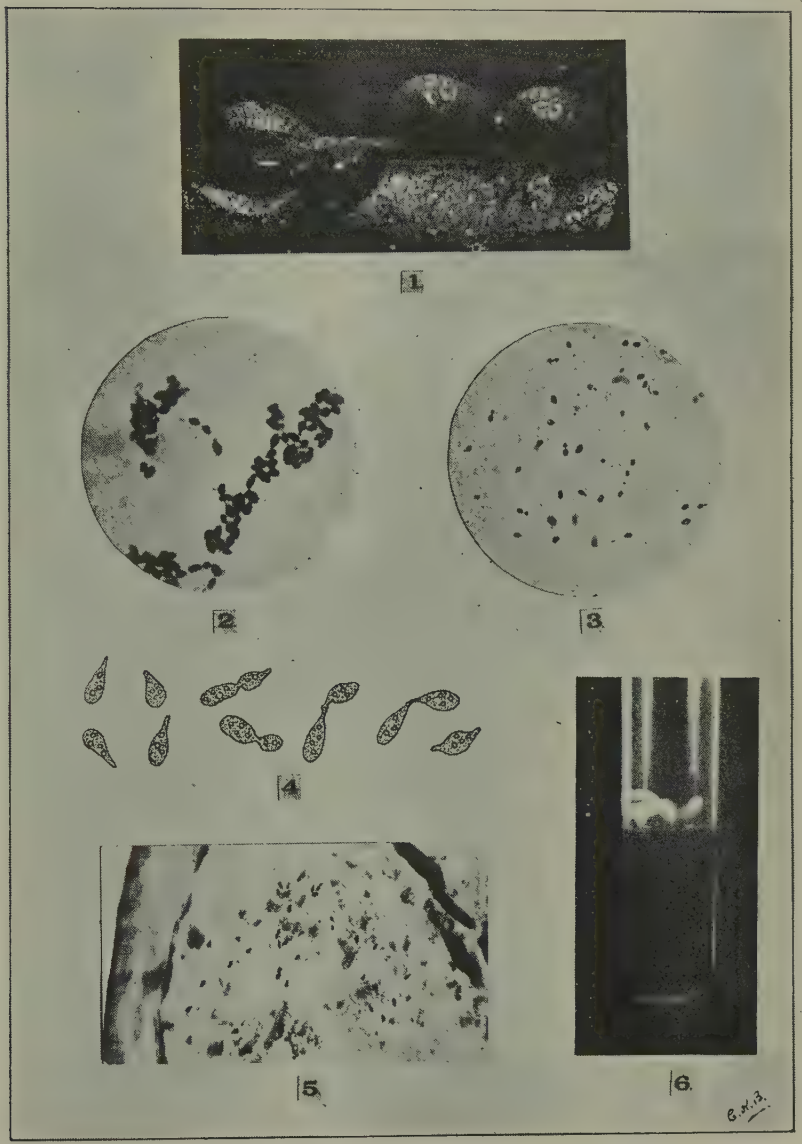

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PLATE IV.

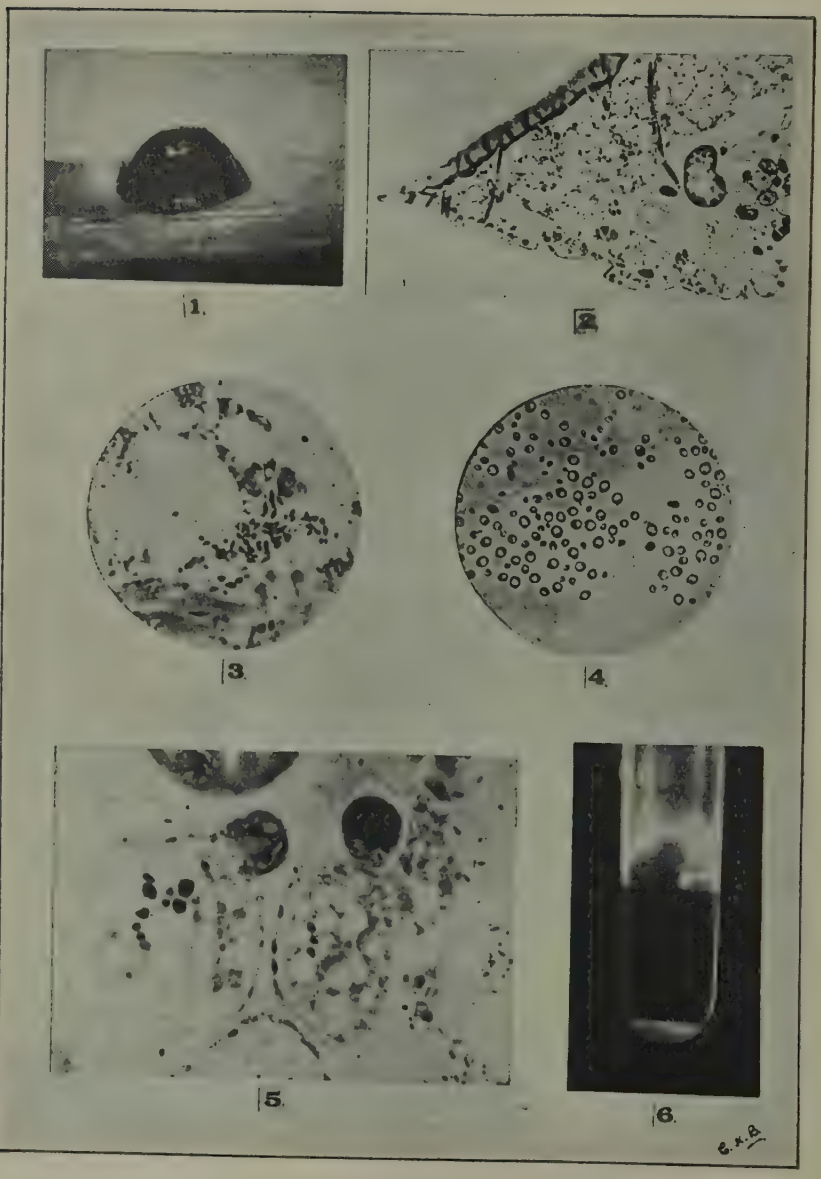

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PLATE V.

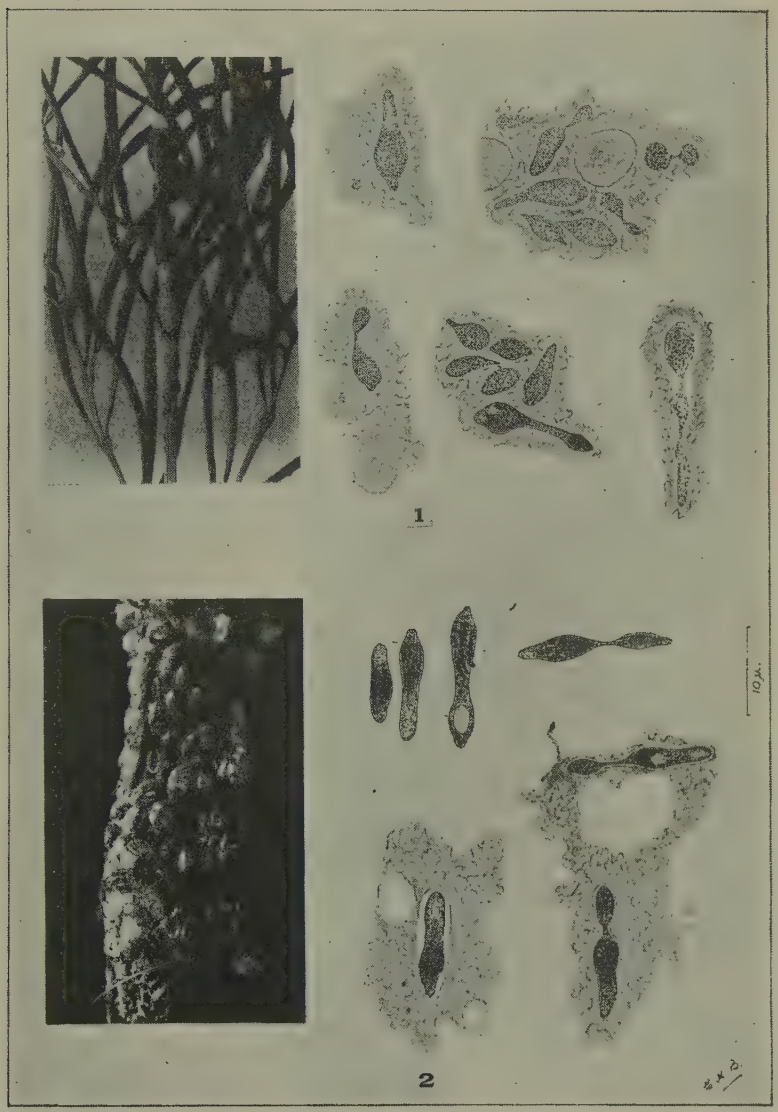

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PLATE VI.

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PLATE VII.

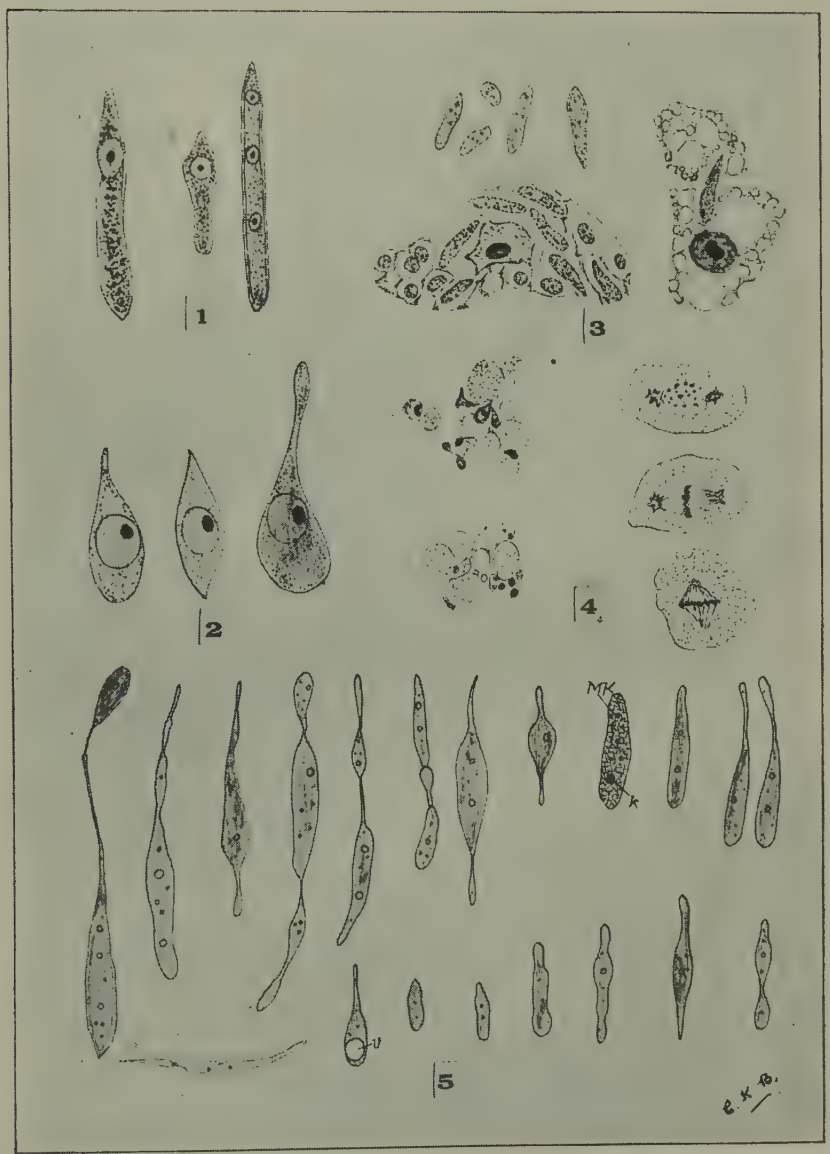

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PLATE VIII.

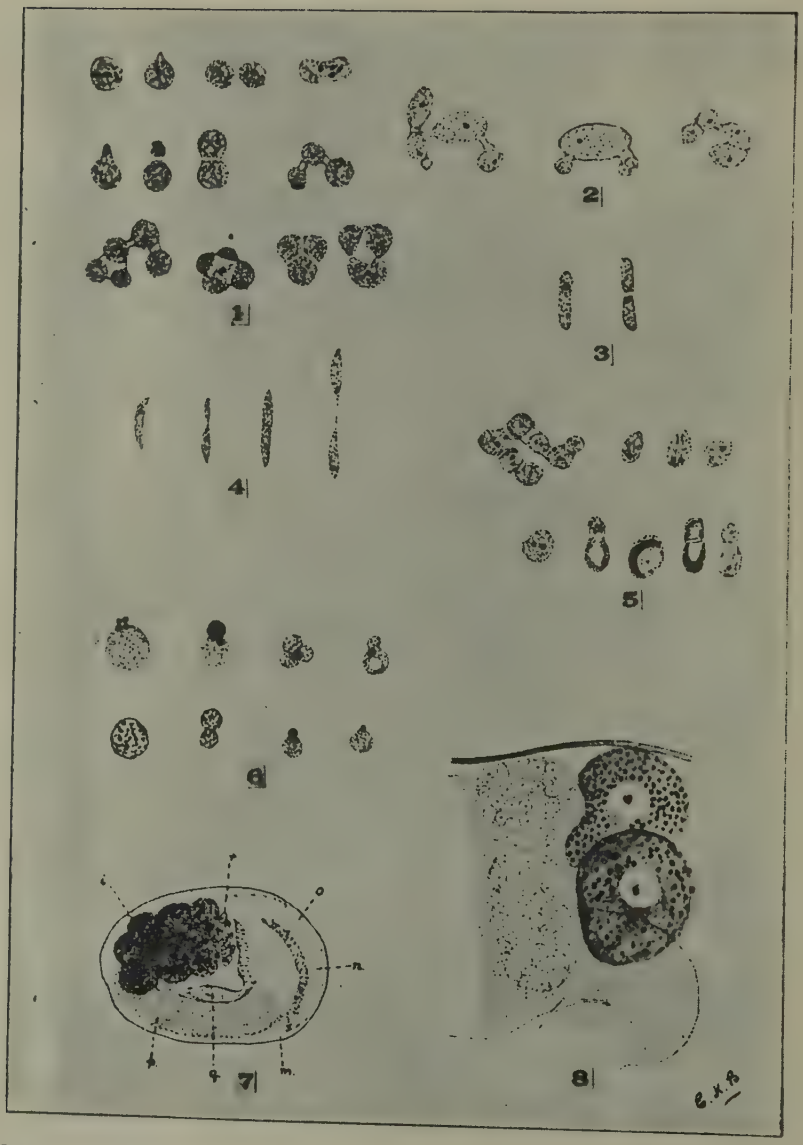

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PLATE IX.

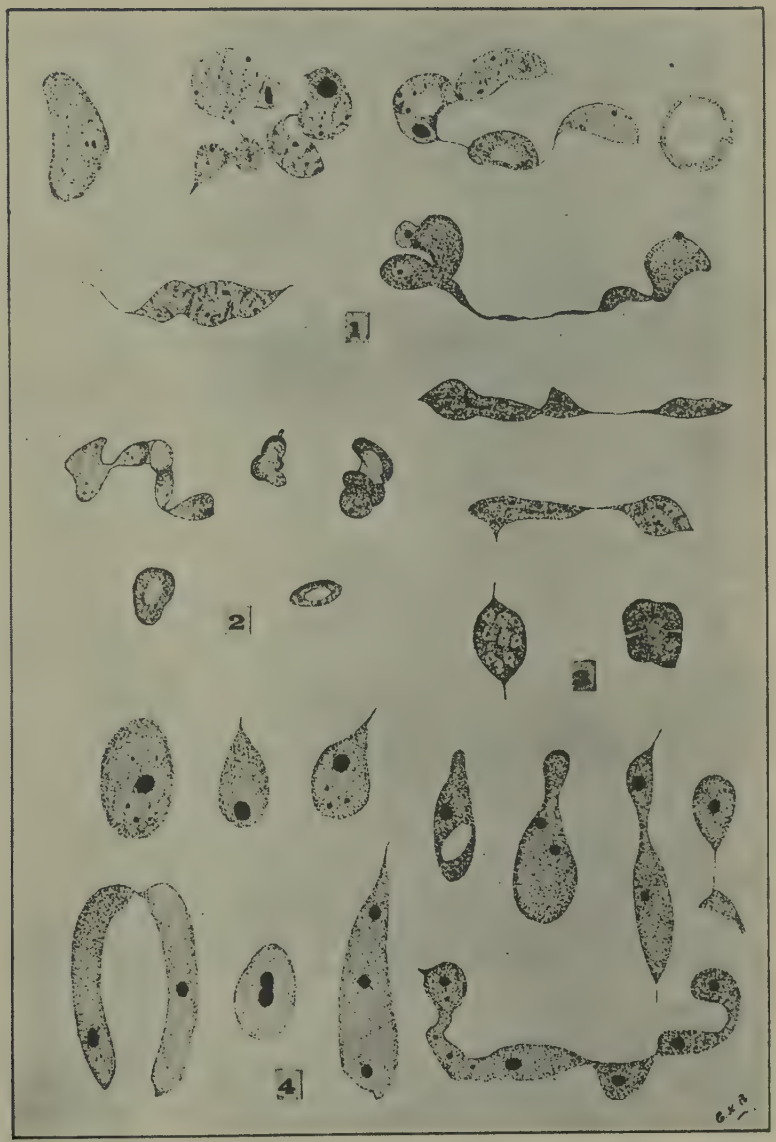

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PLATE X.

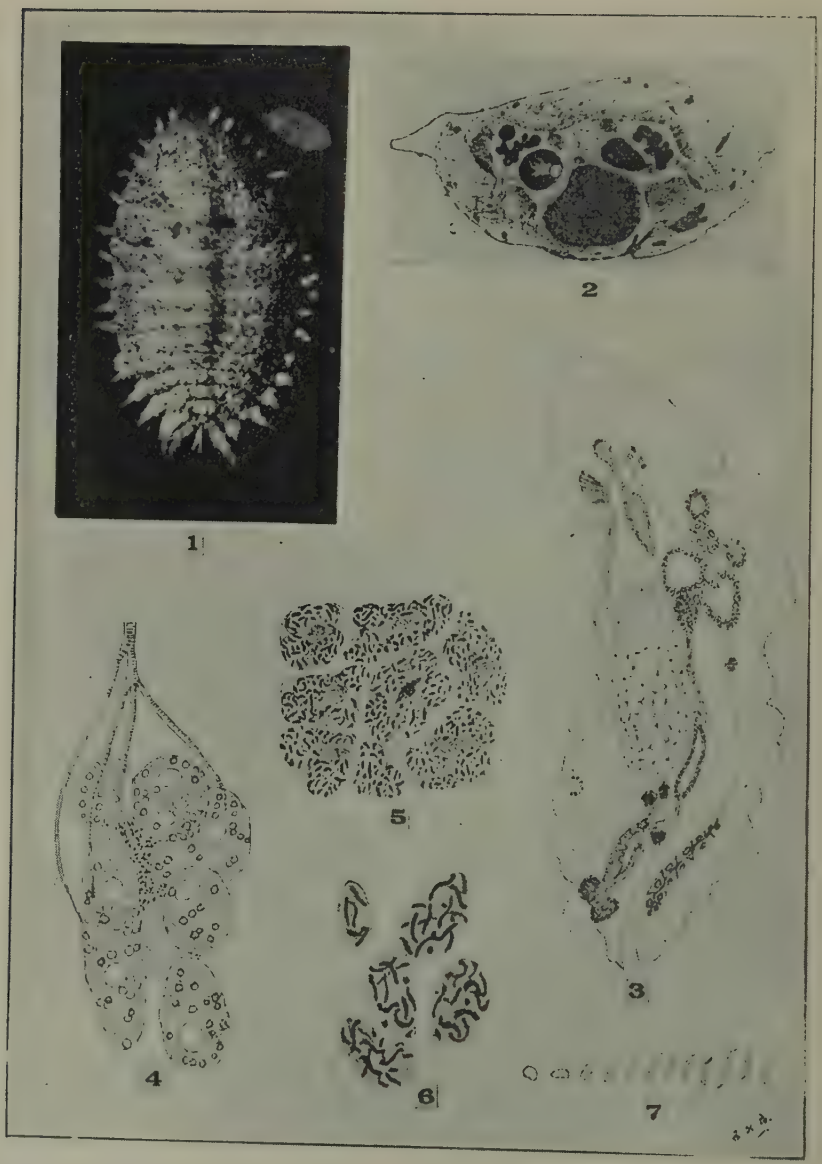

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PLATE XI.

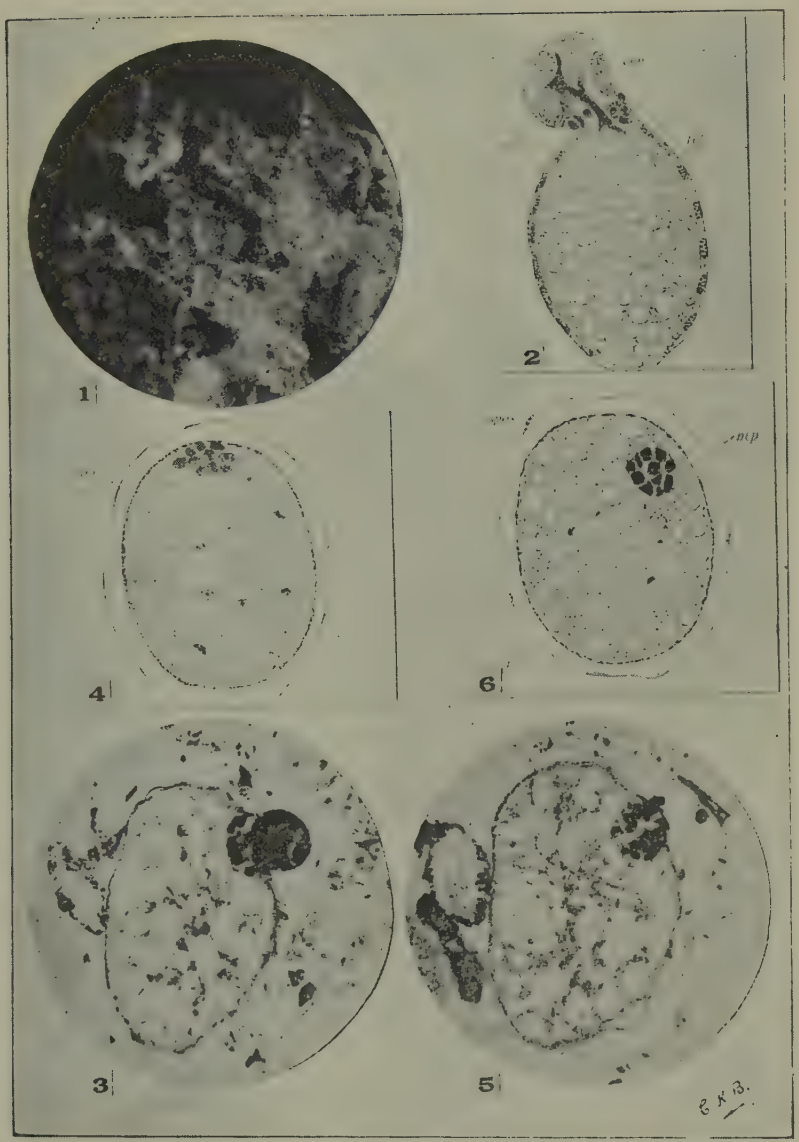

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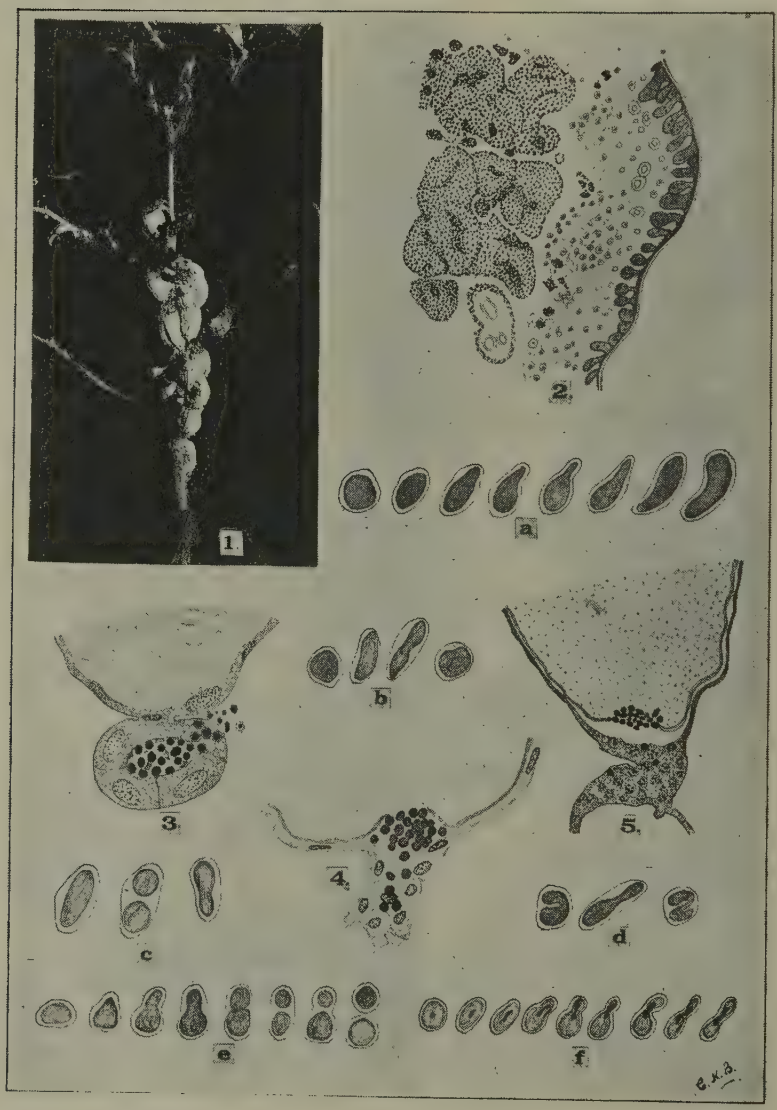
PLATE XII.



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Suid-Afrikaanse Dacryomycetaceae, Tremellaceae en Auriculariaceae.

DEUR

P. A. VAN DER BYL, M.A., D.Sc., Professor vir Mikologie en Fitopatologie aan die Uniwersiteit van Stellenbosch.

(A summary in English appears at the end of the article.)

Ons kennis van die fungusse of swamme wat in Afrika voorkom, is nog baie onvolledig; en die studie daarvan is vir Afrikaanse studente minder gemaklik omdat hul beskrywings in verskillende joernale, meestal buitelandse, gepubliseer is, en dus nie binne die bereik van die meeste Afrikaanse studente is nie. Om Afrikaanse studente en versamelaars van dié laere plante in die geleentheid te stel om dié wat in Afrika voorkom, te bestudeer, en ons wetenskaplike kennis van hul te vermeerder, probeer ons om 'n goed-verteenwoordigende versameling van hul bymekaar te maak en te bewaar vir studiedoeleindes.

Verder is dit ons plan om sommige van hul famielies en geslagte sistematies oor te werk, en hoop ons om van tyd tot tyd korte beskrywings van die soorte wat in Afrika voorkom, in hierdie *Annale* te publiseer.

In hierdie artiekel behandel ons die vir die skrywer tot dusver bekende Afrikaanse geslagte en soorte van die famielies *Dacryomycetaceae, Tremellaceae,* en *Auriculariaceae.*

Voor ons oorgaan om die famielies sistematies te behandel, is dit miskien wenslik om sommige van die terme wat ons sal gebruik, te verklaar.

Die fungusse wat tot die bogenoemde famielies behoor, vorm in die meeste gevalle min of meer jellieagtige massas of gewasse op dooie stompe en takke.

Dié jellieagtige massas is die vrugliggame van die fungusse en hul is opgebou uit fungus-drade of hiefes wat mikroskopies klein is.

Na die oppervlakte van die vrugliggame loop die hiefes uit in gespesialiseerde hiefes wat die *spore* of voortplantingskieme dra en *basidieë* genoem word.

Die basidieë lê naas mekaar en vorm so 'n laag of vlies op die oppervlakte van die vrugliggaam, en dit word die *kiemvlies* genoem.

Die drie bogenoemde famielies word van mekaar onderskei aan hul basidieë.

Die *Dacryomycetaceae* het basidieë wat in twee arme gevurk is, en elkeen van die arme versmal tot 'n sterigme wat één enkele spoor dra (Fig. 1).

Die *Tremellaceae* het basidieë wat in die lengte kruisvormig gedeel is en elkeen van die verdelinge loop uit tot 'n sterigme wat één spoor dra (Fig. 2).

By die *Auriculariaceae* is die basidieë deur dwarsmure in vier selle gedeel en elkeen van hul dra 'n steeltjie of sterigme en elke sterigme dra weer één spoor (Fig. 3).

Ná dié algemene inleiding en verduideliking van sommige van die wetenskaplike terme, sal ons oorgaan om die drie famielies sistematies te behandel en sal ons korte beskrywings gee van die geslagte en soorte wat uit Afrika bekend is.

Die beskrywings is gemaak van eksemplare in my eie kolleksie aan die Uniwersiteit alhier. Vir 'n paar soorte, waarvan ek nie eksemplare gesien het nie, neem ek met erkenning die beskrywings van ander outeurs oor.

Die nommers tussen hakies refereer na my herbarium waar die eksemplare bewaar is.

Ek is dank verskuldig aan mnr. C. G. Lloyd, van Amerika, vir hulp verleen by die benoeming van die fungusse.

A. DACRYOMYCETACEAE.

Basidieë silindries, vurksgewyse in twee arme gedeel, wat hul bo tot sterigmes versmal, elkeen waarvan een enkele en betreklik groot spoor dra.

Sleutel tot die Geslagte.

Vrugliggaam sittend of sonder steel, kussingvormig.	*I. Dacryomyces.*
Vrugliggaam min of meer met 'n steel, silindries, tregter-, spatel-, of oorvormig: Kiemvlies bedek die hele oppervlakte van die vrugliggaam.	*II. Calocera.*
Kiemvlies net op 'n deel van die oppervlakte.	*III. Guepinia.*

I. DACRYOMYCES, NEES.

Vrugliggaam jellieagtig, kussingvormig, soms syfings plat gedruk, sittend; kiemvlies glad of geplooi en harsingvormig; basidieë met lang sterigmes wat tot punte uitloop; spore kleurloos, dwarsgedeel.

Sleutel tot die Soorte.

Kiemvlies eiergeel van kleur. 1. *D. australis.*
Kiemvlies geelbruin van kleur. 2. *D. deliquescens.*

1. *Dacryomyces australis,* Lloyd.

Vrugliggaam jellieagtig, 2 cm. hoog, harsingvormig, met geplooide golwende lobbe; kiemvlies eiergeel van kleur en op 'n bleek voetstuk; gedroogde vrugliggaam donker van kleur en met 'n harpuisagtige voorkoming; spore effens gebuie, deur dwarsmure in 2 tot 9 selle gedeel, glad, 16—20 x 7—8 μ.

Op ou hout, Pietermaritzburg (595 en 627). Herkenbaar aan sy eiergeel kiemvlies. Meer eksemplare is nodig om te besluit of die bleek voetstuk 'n konstante eienskap is.

2. *Dacryomyces deliquescens,* (Bull.) Duby.

Plante groei saam met en naas mekaar; vrugliggaam jellieagtig, geelbruin, kussingvormig of in die omtrek verleng, effens berimpel, word meer plat en harpuisagtig wanneer droog, 2—5 mm. lank x 1—3 mm. breed x 1—3 mm. hoog; spore effens gebuie, glad, word deur dwarsmure in 2—4 selle gedeel, 12 x 4—5 μ.

Op ou vrot hout, Klapmuts (211); Knysna deur mej. A. V. Duthie op ou hout en op dennebolle.

Dacryomyces digressus is deur mej. A. V. Duthie gekollekteer op ou hout by Knysna, maar ek het nie eksemplare daarvan vir ondersoek gehad nie. Volgens beskrywing deur Lloyd is hy dun, harsingvormig, lig vuilgeel van kleur, en met spore 12 x 6 μ.

II. CALOCERA, Fries.

Vrugliggaam regopstaande, silindries, jellie-leeragtig tot kraakbeenagtig; kiemvlies oor die hele omtrek van die vrugliggaam, glad; spore silindries, kleurloos.

Calocera cornea (Batsch.) Fries.

Plante groei saam met mekaar; vrugliggaam regop, elsvormig, effens gebuie, versmal na onder, kraakbeenagtig,

horingagtig wanneer droog, lemoengeel, droog rooiagtig, .5—1 c.m. hoog; kiemvlies oor die hele omtrek van die vrugliggaam, glad; spore silindries, effens gebuie, 8—24 x 5—8 μ, deur dwarsmure in 3 tot 7 selle gedeel voor hul ontkiem.

Op vrot stomp, Pietermaritzburg (591 en 623). Die meeste spore meet 8—12 x 5 μ; verskeie van 24 x 8 μ en deur dwarsmure in sewe selle gedeel was ook teenwoordig. Moontlik vergroot sommige van die eersgenoemde en word meersellig voor hul ontkiem.

III. GUEPINIA, Fries.

Vrugliggaam regopstaande, min of meer met 'n steel, jellieagtig, spatel- of tregtervormig; kiemvlies uitsluitlik aan die onderkant van die vrugbare gedeelte, glad of geplooi; spore kleurloos, langwerpig, word tweesellig.

Groei op ou hout.

Sleutel tot die Soorte.

Vrugliggaam oranjegeel; kiemvlies glad of onduidelik geplooi. 1. *G. spatularia.*

Vrugliggaam liggeel; kiemvlies geplooi; die plooie lyk na verhewe plaatjies. 2. *G. agariciformis.*

1. *Guepinia spatularia* (Schw.) Fries.

Vrugliggaam met 'n steel, oranjegeel, jellieagtig, spatelvormig, regopstaande, groei òf alleen òf in groepe, buigsaam, hard wanneer droog; steel fynbehaar, 1 mm. diam. x 2—3 mm. lank; spatelvormige gedeelte .5—.6 mm. diam., boonste oppervlakte min of meer konkaaf, onderste min of meer konveks; kiemvlies glad of effens en onduidelik geplooi; basidieë gevurk; spore kleurloos, gebuie, word tweesellig, 8—12 x 4—4.5 μ diam.

Op dooie stomp, Knysna (732); ook by Durban, Natal.

2. *Guepinia agariciformis,* Lloyd.

Plante groei dig op mekaar in groepe; vrugliggaam jellieagtig, liggeel, spatel-tregtervormig, 1 cm. hoog; kiemvlies duidelik geplooi en die plooie lyk na verhewe plaatjies; spore kleurloos, gebuie, tweesellig, 19 x 4 μ.

Op ou hout, Pietermaritzburg (770). Waarskynlik is *G. agariciformis* maar net 'n buitengewone vorm van *G. spatularia;* die hoofverskil is in die kiemvlies.

B. TREMELLACEAE.

Basidieë min of meer bolvormig, in die lengte kruisvormig in 2 tot 4 parte gedeel.

Sleutel tot die Geslagte.

Vrugliggaam vorm 'n dun kors, kiemvlies met skerp korrels bedek.	I. *Heterochaete.*
Vrugliggaam dik, jellieagtig, kiemvlies het nie die bogenoemde korrels nie:	
Oppervlakte van vrugliggaam met stippeltjies bedek; basidieë ietwat gekleur.	II. *Exidia.*
Bogenoemde stippeltjies afwesig; basidieë kleurloos.	III. *Tremella.*

I. HETEROCHAETE, Pat.

Heterochaete andina, Pat, en Lagerh. (Fig. 4). Vrugliggaam korsagtig, kruipend, uitgesprei, dun, 40—80 μ dik, opgebou uit kleurlose fungus hiefes wat dig in mekaar gevleg is; oppervlakte vaalgeel, bedek met silindriese, min of meer punterige korrels, 60—280 μ (of meer) lank x 48—60 μ diam.; korrels opgebou uit 'n sentrale bundel van geïnkrusteerde fungus hiefes; basidieë in die lengte gedeel, 12—6 x 6—9 μ; spore kleurloos, effens gebuie, 12 x 8 μ.

Op dooie tak, Durban (629); op dooie tak van Euphorbia pulcherrima (698).

Die plant kan gemaklik van ander plante van die famielie onderskei word aan sy korrelagtige voorkoming en manier van groei.

II. EXIDIA, Fries.

Vrugliggaam jellieagtig, sag; oppervlakte met stippeltjies bedek; basidieë effens gekleur; spore kleurloos, betreklik lank.

Sleutel tot die Soorte.

Vrugliggaam lig van kleur, byna wit.	1. *E. Duthiei.*
Vrugliggaam donker van kleur:	
'n Menigte dun, gekleurde buisies (glœosistidieë) kort onder die oppervlakte van die vrugliggaam.	2. *E. caespitosa.*
Bogenoemde gekleurde buisies afwesig.	3. *E. purpureo-cinerea.*

1. *Exidia Duthiei,* Lloyd.

(Beskrywing oorgeneem van Lloyd.)

Vrugliggaam byna wit en met 'n ligpers tint, min of meer plat, en met gevoude en golwende lobbe; stippeltjies op die oppervlakte van vrugliggaam van dieselfde ligte kleur; oppervlakte skurf onder die mikroskoop; basidieë bolvormig, kleurloos, diep ingesink; spore kleurloos, silindries, gebuie, 8 x 16 μ, inhoud korrelagtig.

Gekollekteer deur mej. A. V. Duthie.

Die plant word van ander soorte van sy geslag onderskei aan sy ligte kleur en stippeltjies van dieselfde kleur.

2. *Exidia caespitosa,* Lloyd.

Plante groei saam dig aanmekaar; vrugliggaam jellieagtig, 2—5 mm. dik, vaalpurper; oppervlakte dig bedek met klein stippeltjies; ingesink kort onder die oppervlakte, is daar baie dun, gekleurde buisies (glœosistidieë).

Op ou stompe, Stellenbosch (481); by Knysna deur mej. A. V. Duthie. Gemaklik herkenbaar aan die gekleurde buisies kort onder die oppervlakte van die vrugliggaam.

3. *Exidia purpureo-cinerea,* Kalch. (Fig. 5.)

Vrugliggaam jellieagtig, vaalbruin, swart wanneer droog, 2—6 mm. dik; oppervlakte dig bedek met 'n menigte klein stippeltjies; basidieë bolvormig, liggekleur, in die lengte gedeel; spore kleurloos, niervormig, 12—20 x 6 μ, inhoud korrelagtig; gekleurde buisies afwesig.

Op ou takke, Stellenbosch (480); op dooie Eucalyptus, Pietermaritzburg (174); op dooie stomp van Pinus, Groot Drakenstein (772); op dooie tak van Quercus, Stellenbosch (489).

III. TREMELLA (Dill.) Fries.

Vrugliggaam sag, jellieagtig tot wasagtig, blaarvormig of harsingvormig; basidieë oor die hele vrugliggaam, in die lengte gedeel; spore meestal kleurloos, bolvormig tot ellipties, klein in vergelyking met die spore van *Exidia.*

SLEUTEL TOT DIE SOORTE.

Vrugliggaam wit.	1. *T. fusiformis.*
Vrugliggaam geelagtig :	
Spore min of meer bolvormig :	
Spore 3.5—4 μ diam.	2. *T. microspora.*
Spore 8 μ diam.	3. *T. crassa.*
Spore ellipties :	
Vrugliggaam liggeel.	4. *T. lutescens.*
Vrugliggaam helder lemoengeel.	5. *T. mesenterica.*

1. *Tremella fusiformis,* Berk. (Fig. 6.)

Vrugliggaam blaarvormig, 1·5—3 cm. hoog, jellieagtig, buigsaam, staan regop òf alleen òf in groepe by mekaar, wit, herhaaldelik gelob; lobbe dun, plat, golwend; basidieë min of meer bolvormig, in die lengte gedeel; spore (volgens Burt) kleurloos, 5—6 x 4—4.5 μ.

Op dooie hout, Durban (605 en 630); Knysna (734).

Die plant kan herken word aan sy wit, rosetagtige, gelobde en golwende gewas.

2. *Tremella microspora,* Lloyd.

(Beskrywing oorgeneem van Lloyd.)

Vrugliggaam dun, blaarvormig; as hy vars is, is hy roomkleurig en in dele rooi (Duthie), uitgeweek ná hy gedroog was, is hy bruingeel; basidieë bolvormig, kruisvormig gedeel; spore kleurloos, min of meer bolvormig, 3.5—4 μ diam.

Gekry by Jonkershoek, Stellenbosch, deur mej. A. V. Duthie.

Die soort is herkenbaar aan sy klein, bolvormige spore.

Lloyd sê dat die plant die algemene voorkoming het van *Auricularia auricula-Judae.*

3. *Tremella crassa,* Lloyd.

Vrugliggaam jellieagtig 3—5 cm. diam. x .5—1 cm. dik, swart wanneer droog en liggeel wanneer uitgeweek; oppervlakte effens harsingvormig en bedek met trossies van klein korreltjies; spore (volgens Lloyd) bolvormig, 8 μ diam.

Op dooie hout, Knysna (725). Herkenbaar aan sy spore.

4. *Tremella lutescens,* Persoon.

Vrugliggaam jellieagtig, blaarvormig; blaartjies heen en weer gedraai, golwend, 4.5 cm. hoog x 2—3 cm. breed x 1 mm. (of minder) dik; roomkleurig wanneer vars en donkerder geel wanneer droog; basideë bolvormig, 8 μ diam., in die lengte gedeel, 16—40 μ lank; spore (volgens Bourd. en Galz.) 10—16 x 7—10 μ.

By Knysna deur mej. A. V. Duthie, 142 (1141). Die soort is na aan *T. mesenterica* maar ligter van kleur. Meestal is hy blaarvormig terwyl *T. mesenterica* gewoonlik harsingvormig is maar soms ook blaarvormig.

5. *Tremella mesenterica,* (Retz.) Fries.

Vrugliggaam jellieagtig, horingagtig wanneer droog, .5—1.5 cm. dik; oppervlakte helder lemoengeel, golwend-geplooi, harsingsvormig, basidieë 16—20 μ diam., bolvormig; spore (volgens Rea) kleurloos, breed-ellipties, 13—14 x 7—8 μ.

Stellenbosch deur J. A. Brink (1055). Verskil van *T. lutescens* in sy helderder geel kleur en vorm.

C. AURICULARIACEAE.

Basidieë silindries, deur dwarsmure in vier selle gedeel en elkeen van die selle dra 'n dun, lang sterigme.

AURICULARIA, Bull.

Vrugliggaam jellie-leeragtig, kraakbeenagtig wanneer droog, halfrond-, koppie-, of oorvormig, sittend of min of meer met 'n steel; basidieë soos onder die famielie genoem. Groei op hout.

Sleutel tot die Soorte.

Kiemvlies bepaald porieagtig. 1. *A. delicata.*

Kiemvlies nie bepaald porieagtig nie:

Boonste oppervlakte van vrugliggaam geel. 2. *A. flava.*

Oppervlakte nie geel nie:

Boonste oppervlakte bedek met bundels growwe, bruin hare, wat swart is as hul nat is. 3. *A. squamosa.*

Sulke growwe, bruin hare afwesig:

Kiemvlies glad. 4. *A. polytricha.*

Kiemvlies geplooi:

Boonste opervlakte geelbruin, behaar; kiemvlies loodkleurig, ribvormig geplooi. 5. *A. lobata.*

Boonste oppervlakte rooibruin, fyndonsig behaar; kiemvlies vaalagtig, aarvormig geplooi. 6. *A. auricula-Judae.*

Boonste oppervlakte donkerbruin, gevoor, donsig behaar; kiemvlies geelbruin, netvormig geplooi. 7. *A. ornata.*

1. *Auricularia delicata* (Fries), Hennings.

Vrugliggaam jellieagtig, buigsaam, 2—2.5 x 3.5 x .2 cm., oorvormig of skulpvormig, geelbruin tot donkerbruin, wanneer droog, dun (·5 mm. of minder) en ietwat deurskynend; meestal vas aan die voorwerp waarop hy groei met 'n kort steeltjie, wat van sy boonste oppervlakte, en dig by sy rand ontstaan; boonste oppervlakte fyn behaar, ferweelagtig; kiemvlies (onderste oppervlakte) porieagtig; porieë 1—2 mm. diam. x 1 mm. diep; basidieë kronkelend, dwars gedeel, 30—45 x 4·5—5 μ; spore kleurloos, eensellig, gebuie, 8—12 x 4—6 μ.

Op vrot hout, Eshowe, Soeloeland (502 en 31). Herkenbaar aan sy bepaald porieagtige kiemvlies.

2. *Auricularia flava,* Lloyd.

Vrugliggaam jellieagtig, teruggebuig; hoed gehalveerd, buigsaam, dun, 2 x 4 x .05 cm.; oppervlakte geel (ou goud) wanneer nat, en vaalbruin wanneer droog, bedek met fyn, kleurlose haartjies; kiemvlies geel, netvormig geplooi; plooie ook by gedroogde eksemplare sigbaar; weefsel tussen oppervlakte en kiemvlies kleurloos; spore min, kleurloos, 12 x 4 μ konidieë bolvormig, 3—4 μ.

Gekry by Lobatsi deur mej. B. Breach (426). Die plant word aan sy geel kleur gemaklik van die ander soorte onderskei. Die kiemvlies is geplooi soos by *A. ornata,* maar by *A. flava* bly die plooie ook by gedroogde eksemplare sigbaar terwyl dit nie die geval by *A. ornata* is nie.

3. *Auricularia squamosa,* Pat.

Vrugliggaam kruipend-teruggebuig, leeragtig, buigsaam, 5 x 6 cm. (of minder) x .5 mm. dik; oppervlakte bedek met bundels van lang, bruin gekleurde haartjies, swart as dit nat is; kiemvlies glad, tabakbruin, word dikwels swart wanneer droog; basideë 36—48 x 3.5—4 μ; spore kleurloos, 6—12 x 3.5—4 μ.

Op ou hout, Soeloeland (251); Durban (250). Die plant kan gemaklik herken word aan die bundels lang, bruingekleurde, haartjies, wat sy oppervlakte bedek. Hy is die grootste en ook die grofste soort van die hele geslag.

4. *Auricularia polytricha* (Mont.) Pat.

Vrugliggaam buigsaam, halfrond-, koppie-, of oorvormig, 2—6 cm. x 1 mm. (of minder), sittend of agter tot 'n kort steeltjie uitgegroei; rand golwend; oppervlakte dig bedek met lang, vaal tot bruinagtige hare; kiemvlies vaalagtig tot bruin- of swartagtig, glad; spore (Saccardo) kleurloos, min of meer niervormig, 20—22 x 8 μ.

Dooie stompe, Durban (185, 186, en 332). Die plant is na aan *A. auricula-Judae* maar verskil deurdat hy dikker en meer harig is en ook deurdat sy kiemvlies glad is.

5. *Auricularia lobata* (Sommerf.) Quél. (Fig. 7.)

Vrugliggaam kruipend-teruggebuig tot amper heeltemal kruipend, gelob, jellieagtig, buigsaam, .5—2.5 mm. dik; oppervlakte geelbruin, donsig behaar; kiemvlies loodkleurig (vaalbruin), ribvormig geplooi, wanneer droog net geaar; basidieë 28—48 x 3.5—4 μ.

Op dooie stomp Viktoria-Valle, Rhodesië (675). Herkenbaar aan sy loodkleurige, geribde kiemvlies, wat by gedroogde eksemplare net geaar is.

6. *Auricularia auricula-Judae* (Linn.) Schroet. (Fig. 8.)

Vrugliggaam buigsaam, jellieagtig tot kraakbeenagtig, halfrond, koppie-, of oorvormig, 3.5—5 cm. diam. x .5 mm. (of minder), geplooi, deurskynend; oppervlakte rooibruin, donsig met fyn verspreide haartjies; kiemvlies aaragtig geplooi, vaalagtig van kleur; gedroogde eksemplare is bruin tot swart; basidieë 40 x 4 μ, dwars gedeel; spore kleurloos, gebuie, 12—16 x 6—8 μ.

Op ou hout, Durban (333, 616, 624 en 737). Die fungus varieer baie, en party vorme is as aparte soorte beskryf geword. *A. auriformis,* Sacc., b.v. wat ek in die Provinsie Natal gekry het (913), is maar 'n ligter gekleurde vorm van *A. auricula-Judae. A. auricularis,* Fr. wat ek by Durban, Natal gekry het (333), is ook maar 'n ligter gekleurde en minder harige vorm van dieselfde fungus.

7. *Auricularia ornata,* Persoon.

Vrugliggaam kruipend-teruggebuig tot byna geheel kruipend, jellieagtig, buigsaam, .5—2 mm. dik; oppervlakte donkerbruin, konsentries gevoor, donsig behaar; kiemvlies geelbruin (ligter dan oppervlakte), netvormig geplooi; gedroogde eksemplare donkerbruin en kiemvlies net met enige are; basidieë dwars gedeel, 28 x 40 x 3.5—4 μ; spore kleurloos, 7.5 x 4 μ.

Op dooie hout, Durban (588).

SUMMARY IN ENGLISH.

The paper is a systematic account of the *Dacryomycetaceae, Tremellaceae* and *Auriculariaceae* known to occur in South Africa.

The specimens on which the descriptions are based are with a few exceptions preserved in the writer's herbarium at the University of Stellenbosch. The followning genera and species of the above named families are described or mentioned:

Dacryomycetaceae.	*Tremellaceae.*	*Auriculariaceae.*
Calocera cornea.	Exidia caespitosa.	A auricularis.
Dacryomyces australis.	E. Duthiei.	A. auriformis.
D. deliquescens.	E. purpureo-cinerea.	A. auricula-Judae.
D. digressus.	Heterochaete andina.	A. delicata.
Guepinia agariciformis.	Tremella crassa.	A. flava.
G. spatularia.	T. fusiformis.	A. lobata.
	T. lutescens.	A. ornata.
	T. mesenterica.	A. polytricha.
	T. microspora.	A. squamosa.

VERKLARING BY DIE ILLUSTRASIES.

(Fig. 1—3 is volgens Brefeld.)

Fig. 1. 'n Snit deur die kiemvlies van *Dacromyces deliquescens*. (350-maal vergroot.) *a*, sterigmes; *b*, gevurkte basidieë; *c*, spore.

Fig. 2. 'n Snit deur die kiemvlies van *Tremella lutescens*. (450-maal vergroot). *a*, sterigmes; *b*, kruisvormige gedeelde basidieë; *c*, spore; *d*, konidieë.

Fig. 3. Dwars gedeelde basidie van 'n *Auricularia*. (300-maal vergroot). *a*, sterigmes; *b*, basidie deur dwars mure gedeel; *c*, spoor.

Fig. 4. *Heterochaete andina*, Pat. en Lagerh.

Fig. 5. *Exidia purpureo-cinerea*, Kalch.

Fig. 6. *Tremella fusiformis*, Berk.

Fig. 7. *Auricularia lobata* (Sommer.) Quél.

Fig. 8. *Auricularia auricula-Judae* (Linn.) Schroet.

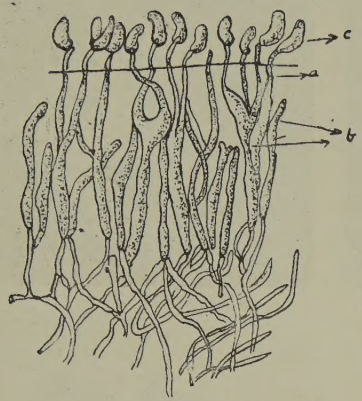

Fig. 1.

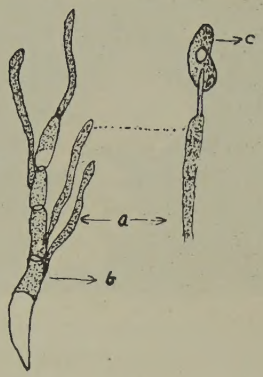

Fig 3

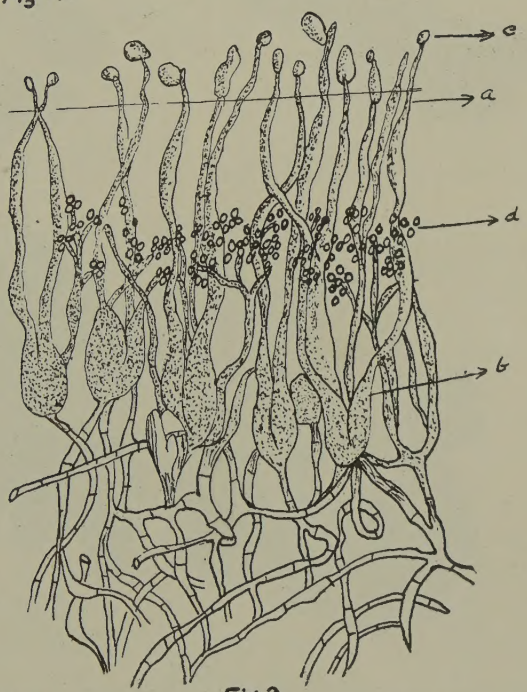

Fig 2.

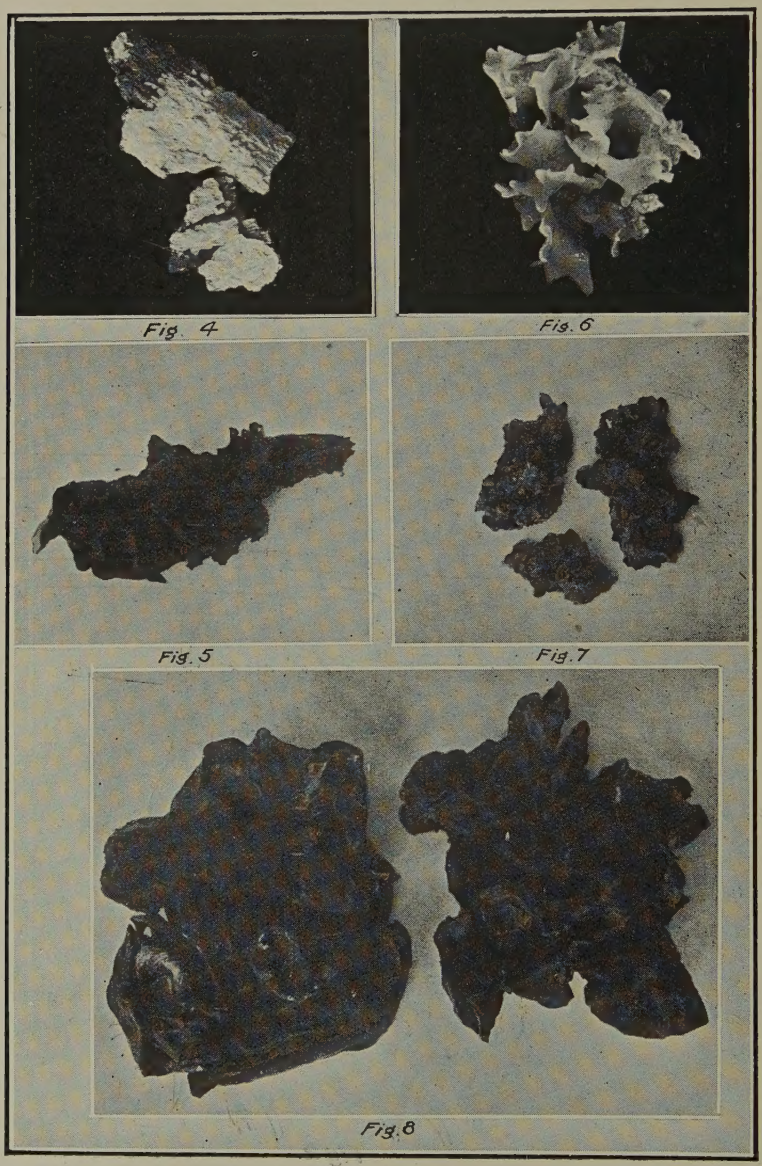

Fig. 4

Fig. 6

Fig. 5

Fig. 7

Fig. 8